

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
7 February 2002 (07.02.2002)

PCT

(10) International Publication Number  
**WO 02/09735 A2**

(51) International Patent Classification<sup>7</sup>: **A61K 38/00**

(21) International Application Number: PCT/US01/24147

(22) International Filing Date: 31 July 2001 (31.07.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/222,071 1 August 2000 (01.08.2000) US

(71) Applicant (for all designated States except US): **UNIVERSITY OF WASHINGTON** [US/US]; Office of Technology Transfer, Suite 200, 1107 NE 45th Street, Seattle, WA 98105-4631 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **BORNSTEIN, Paul** [US/US]; 602 - 34th Avenue, Seattle, WA 98112 (US). **KYRIAKIDES, Themis** [CY/US]; 7729 - 45th Avenue S.W., Seattle, WA 98136 (US). **RATNER, Buddy**

[US/US]; 1612 Bigelow Avenue N., Seattle, WA 98109 (US). **GIACHELLI, Cecilia** [US/US]; 3012 154th Street, S.E., Mill Creek, WA 98012 (US). **MARTINSON, Laura** [US/US]; Dexcon Inc., 6725 Meso Ridge Road, San Diego, CA 92121 (US). **SCATENA, Marta** [US/US]; 1825 N. 51st Street, Seattle, WA (US).

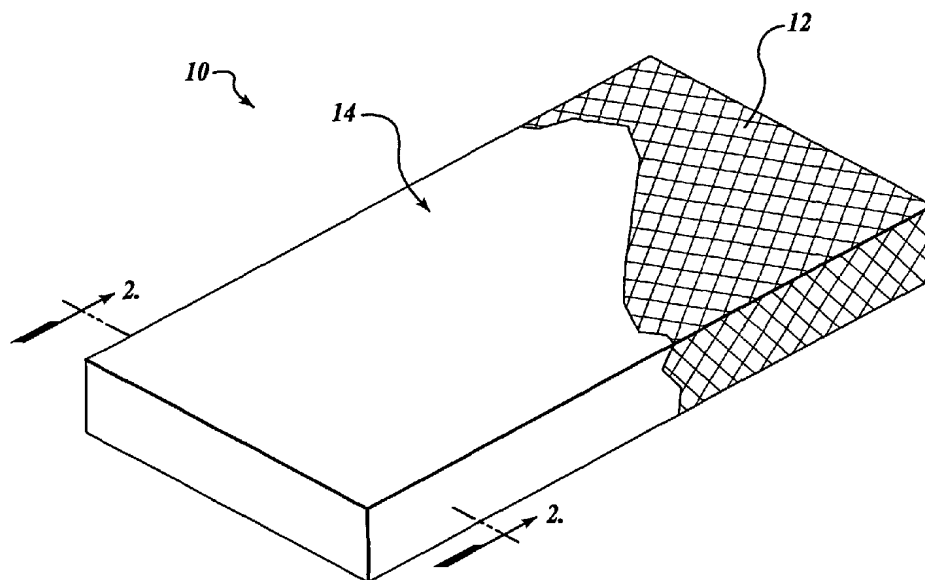
(74) Agent: **McGURL, Barry, F.**; Christensen O'Connor Johnson & Kindness PLLC Suite 2800, 1420 Fifth Avenue, Seattle, WA 98101 (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European

[Continued on next page]

(54) Title: METHODS AND DEVICES TO MODULATE THE WOUND RESPONSE



(57) Abstract: In one aspect, the invention provides methods of modulating the amount and/or biological activity of thrombospondin (2) or osteopontin in an animal. The methods comprise the step of introducing into the animal an amount of osteopontin, and/or a thrombospondin (2) antagonist, effective to modulate the amount or biological activity of thrombospondin (2) or osteopontin in the animal. In another aspect, the invention provides medical devices comprising (a) a device body; and (b) a surface layer attached to the device body, the surface layer including an amount of an agents or antagonist of a matricellular protein sufficient to reduce the foreign body response against the medical device, wherein the medical device is adapted to be affixed to, or implanted within, the soft tissue of an animal.



WO 02/09735 A2



patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

**Published:**

— *without international search report and to be republished upon receipt of that report*

## METHODS AND DEVICES TO MODULATE THE WOUND RESPONSE

### BACKGROUND OF THE INVENTION

Animals exhibit a variety of physiological and biochemical responses at the site of tissue damage or injury. These physiological and biochemical responses are collectively referred to as the wound response. The wound response facilitates the repair or replacement of the damaged or destroyed tissue. In some situations, however, wounded tissue exhibits a chronic wound response that adversely affects the health or well-being of the wounded animal.

The implantation of a medical device into soft tissue elicits a wound response. This type of wound response is called the foreign body response and results in the encapsulation of the implant by a poorly-vascularized, collagenous, capsule that can compromise the function of the implant. In addition, the continued presence of the implant can lead to a chronic inflammatory response that is mediated, in part, by macrophages.

Thrombospondin-2 (TSP2) is a secreted, extracellular matrix glycoprotein with potent anti-angiogenic activity (Bornstein et al., 2000, Matrix Biology 19: 557-568). Osteopontin (OPN) is a secreted, phosphorylated glycoprotein that contains cell adhesion domains (Fisher et al., Genomics 7, 491-502 (1990)). The present inventors have discovered that modulation of the amount and/or biological activity of osteopontin (OPN) and/or thrombospondin 2 (TSP2) in an animal can be utilized to

modulate the wound response, such as the foreign body response to an implanted medical device.

#### SUMMARY OF THE INVENTION

In accordance with the foregoing, in one aspect the present invention provides  
5 methods of modulating the amount and/or biological activity of thrombospondin 2 or osteopontin in an animal, the methods comprising the step of introducing into the animal an amount of a molecule, selected from the group consisting of osteopontin and a thrombospondin 2 antagonist, effective to modulate the amount and/or biological activity of thrombospondin 2 or osteopontin in the animal. In this context,  
10 when used with reference to OPN, the term "modulating" means increasing or decreasing the amount and/or biological activity of OPN. In this context, when used with reference to TSP2, the term "modulating" means decreasing the amount and/or biological activity of TSP2. In some embodiments of this aspect of the invention, the amount and/or biological activity of OPN is increased. In some embodiments of this  
15 aspect of the invention, the amount and/or biological activity of TSP2 is decreased.

In another aspect, the present invention provides methods of improving the wound response in an animal, the methods comprising the step of introducing into the animal an amount of a molecule, selected from the group consisting of osteopontin and a thrombospondin 2 antagonist, effective to improve the wound  
20 response in the animal.

In another aspect, the present invention provides methods of reducing the foreign body response in an animal, the methods comprising the step of introducing into the animal an amount of a molecule, selected from the group consisting of osteopontin and a thrombospondin 2 antagonist, effective to reduce the foreign body  
25 response in the animal. Typically, in the practice of the methods of the invention to improve the wound response, and/or to reduce the foreign body response, the amount and/or biological activity of osteopontin is increased, and/or the amount and/or biological activity of thrombospondin-2 is decreased.

The methods of the invention can be used to modulate the wound response in  
30 any situation where modulation of the wound response is desirable, including

situations in which it is desirable to reduce the foreign body response, and including situations in which it is desirable to improve the wound response.

In another aspect, the present invention provides medical devices, each medical device comprises (a) a device body; and (b) a surface layer attached to the device body, the surface layer including an amount of an agonist or antagonist of a matricellular protein sufficient to reduce the foreign body response against the medical device, wherein the device is adapted to be affixed to, or implanted within, the soft tissue of an animal. Thus, the medical devices of the invention are useful in any situation in which it is desired to reduce the foreign body response against an implanted medical device.

#### BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing aspects and many of the attendant advantages of this invention will become more readily appreciated as the same become better understood by reference to the following detailed description, when taken in conjunction with the accompanying drawings, wherein:

FIGURE 1 shows a perspective view of a representative medical device of the invention with a portion of the surface layer removed to expose the underlying device body.

FIGURE 2 shows a transverse cross-section of the medical device of FIGURE 1.

FIGURE 3 shows the porous matrix structure of the surface layer of the representative medical device shown in FIGURE 1.

FIGURE 4 shows a perspective view of a representative medical device of the invention that includes two surface layers disposed one upon the other. The outer surface layer includes osteopontin protein, and the inner surface layer includes an antagonist of thrombospondin 2.

FIGURE 5 shows a perspective view of a representative medical device of the invention that includes a device body, and a surface layer disposed on the device body. The surface layer includes a first area, including a first agonist or first antagonist of a matricellular protein, and a second area, including a second agonist or

second antagonist of a matricellular protein. The first agonist is different from the second agonist, and the first antagonist is different from the second antagonist.

FIGURE 6 shows data showing the extent of vascularization of foreign body capsules formed around devices implanted into mice. The devices were each made from a millipore filter coated with a collagen matrix. The collagen matrices were impregnated with a plasmid including either a TSP2 sense (S), or TSP2 antisense (AS), nucleic acid molecule. P represents devices coated with a collagen matrix that was not impregnated with a plasmid. The devices were implanted into either TSP2-null (-/-) or normal control (+/+) mice. The x-axis shows the number of weeks (two or four) of implantation within a mouse. The y-axis shows the number of blood vessels, per visual field, within each foreign body capsule as viewed under a microscope. "Control" represents implanted millipore filters that were not coated with collagen.

FIGURE 7 shows the number of foreign body giant cells produced at the site of implantation of fixed bovine pericardium samples into either OPN null mice (OPN knockout mice) or normal control mice. The results from seven OPN null mice and seven control mice were measured. The numbers of foreign body giant cells was measured at 14 days and 30 days post implantation.

FIGURE 8A shows the foreign body capsule thickness for polyethylene discs (PE), polyethylene discs coated with tetraglyme (PE glyme), and polyethylene discs coated with tetraglyme to which are covalently attached osteopontin protein molecules (PE glyme OPN). The discs were implanted into mice and the foregoing parameters measured after four weeks.

FIGURE 8B shows the macrophage score (a measure of the number of macrophages in each disc) for the discs described in the legend for FIGURE 8A.

#### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

Unless specifically defined herein, all terms used herein have the same meaning as they would to one skilled in the art of the present invention. The following definitions are provided in order to provide clarity with respect to the terms as they are used in the specification and claims to describe the present invention.

As used herein, the term "wound response" refers collectively to the biochemical and physiological repair processes elicited at the site of wounding in, or on, an animal body, such as by the implantation of a medical device. The wound response is characterized by a transient inflammatory reaction followed by an invasion of collagen secreting fibroblasts and new vasculature formation in the wound bed. These events are followed by granulation tissue formation and remodeling.

The term "matricellular protein" refers to proteins that have the ability to simultaneously interact with a component of the extracellular matrix and a component of the cell surface. Some matricellular proteins can interact with growth factors and/or proteinases. Matricellular proteins function primarily to regulate cell adhesion, movement, and function. Examples of matricellular proteins include TSP1 (Chen et al., Matrix Biology 19: 597-614), TSP2 (Bornstein et al., Matrix Biology 19: 557-568) OPN (Giachelli and Steitz, Matrix Biology 19: 615-622) tenascin-C (Jones and Jones, Matrix Biology 19: 581-596) and SPARC (Brekken and Sage, Matrix Biology 19: 569-580). Each of the foregoing publications are incorporated herein by reference.

The term "foreign body response" refers to a type of wound response in which a poorly-vascularized, collagenous, capsule forms around a structure (such as a medical device) implanted into an animal body.

The phrase "soft tissue of an animal" refers to any animal tissue except bone, nail, or hair. The phrase "soft tissue of an animal" includes, for example, muscle and skin.

The term "hybridize under stringent conditions", and grammatical equivalents thereof, refers to the ability of a nucleic acid molecule to hybridize to a target nucleic acid molecule (such as a target nucleic acid molecule immobilized on a DNA or RNA blot, such as a Southern blot or Northern blot) under defined conditions of temperature and salt concentration. Typically, stringent hybridization conditions are no more than 25°C to 30°C (for example, 10°C) below the melting temperature ( $T_m$ ) of the native duplex. By way of non-limiting example, representative salt and

temperature conditions for achieving stringent hybridization are: 5X SSC, at 65°C, or equivalent conditions; *see generally*, Sambrook et al. *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Press, 1987; Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publishing, 1987.  $T_m$  for nucleic acid molecules greater than about 100 bases can be calculated by the formula  $T_m = 81.5 + 0.41\%(G+C) - \log(Na^+)$ . For oligonucleotide molecules less than 100 bases in length, exemplary hybridization conditions are 5 to 10°C below  $T_m$ . On average, the  $T_m$  of a short oligonucleotide duplex is reduced by approximately  $(500/\text{oligonucleotide length})^\circ\text{C}$ .

The abbreviation "SSC" refers to a buffer used in nucleic acid hybridization solutions. One liter of the 20X (twenty times concentrate) stock SSC buffer solution (pH 7.0) contains 175.3 g sodium chloride and 88.2 g sodium citrate.

The term "sequence identity" or "percent identical" as applied to nucleic acid molecules is the percentage of nucleic acid residues in a candidate nucleic acid molecule sequence that are identical with a subject nucleic acid molecule sequence (such as the nucleic acid molecule sequence set forth in SEQ ID NO:1), after aligning the sequences to achieve the maximum percent identity, and not considering any nucleic acid residue substitutions as part of the sequence identity. No gaps are introduced into the candidate nucleic acid sequence in order to achieve the best alignment.

Nucleic acid sequence identity can be determined in the following manner. The subject polynucleotide molecule sequence is used to search a nucleic acid sequence database, such as the Genbank database (accessible at Website <http://www.ncbi.nlm.nih.gov/blast/>), using the program BLASTN version 2.1 (based on Altschul et al., *Nucleic Acids Research* **25**: 3389-3402 (1997)). The program is used in the ungapped mode. Default filtering is used to remove sequence homologies due to regions of low complexity as defined in Wootton, J.C. and S. Federhen, *Methods in Enzymology* 266: 554-571 (1996). The default parameters of BLASTN are utilized.



The term "sequence identity" or "percent identical" as applied to protein molecules is the percentage of amino acid residues in a candidate protein molecule sequence that are identical with a subject protein sequence (such as the protein sequence set forth in SEQ ID NO:2), after aligning the sequences to achieve the maximum percent identity. No gaps are introduced into the candidate protein sequence in order to achieve the best alignment.

Amino acid sequence identity can be determined in the following manner. The subject protein sequence is used to search a protein sequence database, such as the GenBank database (accessible at web site <http://www.ncbi.nlm.nih.gov/blast/>), using the BLASTP program. The program is used in the ungapped mode. Default filtering is used to remove sequence homologies due to regions of low complexity. The default parameters of BLASTP are utilized. Filtering for sequences of low complexity utilize the SEG program.

The term "antibody" encompasses polyclonal and monoclonal antibody preparations, CDR-grafted antibody preparations, as well as preparations including hybrid antibodies, altered antibodies, F(AB)<sub>2</sub> fragments, F(AB) molecules, Fv fragments, single domain antibodies, chimeric antibodies and functional fragments thereof which exhibit immunological binding properties of the parent antibody molecule. The antibodies can also be humanized.

In one aspect, the present invention provides methods of modulating the amount and/or biological activity of thrombospondin 2 (TSP2) and/or osteopontin (OPN) in an animal. The methods of this aspect of the invention comprise the step of introducing into the animal an amount of a molecule, selected from the group consisting of OPN and a TSP2 antagonist, effective to modulate the amount and/or biological activity of TSP2 or OPN in the animal. In this context, when used with reference to OPN, the term "modulating" means increasing or decreasing the amount and/or biological activity of OPN. In this context, when used with reference to TSP2, the term "modulating" means decreasing the amount and/or biological activity of TSP2. In some embodiments of this aspect of the invention, the amount and/or biological activity of OPN is increased. In some embodiments of this aspect of the

invention, the amount and/or biological activity of TSP2 is decreased. The methods described in the present invention are applicable to any animal, including mammals, such as human beings. The methods of this aspect of the invention can be used to modulate the wound response in any situation where modulation of the wound response is desirable, including situations in which it is desirable to reduce the foreign body response and including situations in which it is desirable to improve the wound response (e.g. improve the rate of wound healing at the site of a cut, abrasion, or burn to soft tissue). In some embodiments, in order to modulate the wound response, the amount and/or biological activity of OPN is increased, and/or the amount and/or biological activity of TSP2 is decreased.

The methods of this aspect of the invention can be used, for example, to improve the wound response (e.g., by increasing the magnitude of one or more of the biochemical and/or physiological and/or physical responses that make up the wound response, and/or reducing the duration of the wound response), such as at the site of a cut, abrasion or burn (e.g. by applying to the cut, abrasion or burn, an article, such as an adhesive strip, that includes an amount of OPN and/or a TSP2 antagonist, that is effective to improve the wound response). Improved wound response is especially important, for example, in a diabetic person, where cuts, abrasions and burns are slow to heal.

The methods of this aspect of the invention can also be used, for example, to reduce the foreign body response, (e.g. by reducing the magnitude of one or more of the biochemical and/or physiological and/or physical responses that make up the foreign body response, and/or reducing the duration of the foreign body response). Representative examples of situations in which it is desirable to reduce the foreign body response include the reduction of the foreign body response against an implanted medical device, thereby prolonging the working lifetime of the implanted device.

Other examples of situations where it is desirable to use the methods of the invention to reduce the foreign body response include: reduction of the foreign body response at the site of an implanted vascular stent, thereby preventing or delaying

restenosis at the location of the stent; and reduction of the foreign body response elicited by tissues or organs implanted into an animal body, thereby promoting the acceptance of the implanted tissue or organ by the host body.

A reduction in the foreign body response is characterized by at least one of the following changes in a component of the foreign body response that occurs as a result of treatment of animal tissue in accordance with the methods of the invention: a decrease in the amount of fibrosis (measured, for example, by a decrease in hydroxy-proline content which indicates the level of collagen in the foreign body capsule); a decrease in the amount of inflammation (measured, for example, by counting the number of inflammatory cells, and the number of foreign body giant cells, in histological sections; or measuring the levels of cytokines, such as interleukin and monocyte chemoattractant protein, in wound extracts by ELISA); an increase in the amount of vascularization of the capsule formed as part of the foreign body response (measured, for example, by visualizing blood vessels in histological sections with anti-PECAM1 antibody and the peroxidase reaction; the number of vessels and their average size are estimated with imaging software such as Metamorph); an increase in the amount of permeability of the capsule formed as part of the foreign body response (measured, for example, as the release of traceable chemicals from implanted devices, or ability of implanted sensors to sense plasma levels of molecules such as glucose); a decrease in the amount of the capsule formed around the foreign body (capsule thickness can be measured from histological sections with the aid of ocular micrometers); and a decrease in the amount of contraction of collagen fibers within the capsule that is formed as part of the foreign body response (measured as tensile strength of the capsule or induced shape change on malleable implants). The decrease, or increase, of any of the foregoing parameters can be a decrease, or increase, relative to the amount of the parameter present before treatment in accordance with the methods of the invention; or a decrease, or increase, relative to the amount of the parameter present in control tissue that is not treated in accordance with the methods of the present invention.

Thus, in one aspect, the present invention provides methods of improving the wound response in an animal, the methods comprising the step of introducing into the animal an amount of a molecule, selected from the group consisting of osteopontin and a thrombospondin 2 antagonist, effective to improve the wound response in the animal. In another aspect, the present invention provides methods of reducing the foreign body response in an animal, the methods comprising the step of introducing into the animal an amount of a molecule, selected from the group consisting of osteopontin and a thrombospondin 2 antagonist, effective to reduce the foreign body response in the animal. Typically, in the practice of the methods of the invention to improve the wound response, and/or to reduce the foreign body response, the amount and/or biological activity of osteopontin is increased, and/or the amount and/or biological activity of thrombospondin-2 is decreased.

Any OPN protein that improves the wound response and/or reduces the foreign body response is useful in the practice of the present invention. OPN proteins useful in the methods of the present invention include naturally purified OPN protein (which may be chemically modified after purification), chemically synthesized OPN protein, and OPN protein produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, insect, mammalian, avian and higher plant cells.

OPN fragments that improve the wound response and/or reduce the foreign body response are also useful in the practice of the present invention. Also, modified OPN proteins, or fragments thereof, that improve the wound response and/or reduce the foreign body response are useful in the practice of the present invention. Modifications can include those that are introduced during or after translation, (e.g., by glycosylation, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand). Modifications also include N-terminal modifications, which result from expression in a particular recombinant host, such as, for example, N-terminal methylation which occurs in certain bacterial (e.g. *E. coli*) expression systems. Modifications also include mutants in which amino acid substitutions are made.

OPN protein, or OPN fragments, can be recovered and purified by any applicable purification method, including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, gel filtration, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography, and high performance liquid chromatography ("HPLC").

The cDNA molecule set forth in SEQ ID NO:1 encodes a representative example of OPN (consisting of the amino acid sequence set forth in SEQ ID NO: 2) useful in the practice of the invention. Other representative examples of useful OPN proteins include OPN proteins that are at least 70% identical to the OPN protein set forth in SEQ ID NO. 2.

The amount and/or biological activity of OPN in an animal can be modulated (for example increased) by any suitable method, such as one or more of the following, representative, methods: the delivery of nucleic acid molecules encoding OPN into the body of an animal; increasing the level of endogenous OPN transcription and/or translation within the body of an animal; delivery of OPN protein (or OPN fragments that retain the ability to modulate the wound response) into the body of an animal by implanting into the body of an animal, or attaching to the body of an animal, a structure comprising OPN, or OPN peptides retaining the ability to modulate the wound response, disposed on a surface of the structure that contacts tissue of the animal body when the structure is implanted therein.

OPN protein, or OPN peptides retaining the ability to modulate the wound response, can be delivered into the body of an animal by any suitable means. By way of representative example, OPN protein, or fragments thereof, can be introduced into an animal body by application to a bodily membrane capable of absorbing the protein, for example the nasal, gastrointestinal and rectal membranes. The protein is typically applied to the absorptive membrane in conjunction with a permeation enhancer. (See, e.g., V.H.L. Lee, *Crit. Rev. Ther. Drug Carrier Syst.*, 5:69 (1988); V.H.L. Lee, *J. Controlled Release*, 13:213 (1990); V.H.L. Lee, Ed., *Peptide and Protein Drug Delivery*, Marcel Dekker, New York (1991); A.G. DeBoer et al., *J. Controlled Release*, 13:241 (1990)). For example, STDHF is a synthetic

derivative of fusidic acid, a steroidal surfactant that is similar in structure to the bile salts, and has been used as a permeation enhancer for nasal delivery. (W.A. Lee, *Biopharm.* Nov./Dec., 22, 1990).

5 The OPN protein, or fragments thereof, may be introduced in association with another molecule, such as a lipid, to protect the protein from enzymatic degradation. For example, the covalent attachment of polymers, especially polyethylene glycol (PEG), has been used to protect certain proteins from enzymatic hydrolysis in the body and thus prolong half-life (F. Fuertges, et al., *J. Controlled Release*, **11**:139 (1990)). Many polymer systems have been reported for protein delivery (Y.H. Bae, 10 et al., *J. Controlled Release*, **9**:271 (1989); R. Hori, et al., *Pharm. Res.*, **6**:813 (1989); I. Yamakawa, et al., *J. Pharm. Sci.*, **79**:505 (1990); I. Yoshihiro, et al., *J. Controlled Release*, **10**:195 (1989); M. Asano, et al., *J. Controlled Release*, **9**:111 (1989); J. Rosenblatt et al., *J. Controlled Release*, **9**:195 (1989); K. Makino, *J. Controlled Release*, **12**:235 (1990); Y. Takakura et al., *J. Pharm. Sci.*, **78**:117 (1989); 15 Y. Takakura et al., *J. Pharm. Sci.*, **78**:219 (1989)).

For transdermal applications, the OPN protein, or fragments thereof, may be combined with other suitable ingredients, such as carriers and/or adjuvants. There are no limitations on the nature of such other ingredients, except that they must be pharmaceutically acceptable and efficacious for their intended administration, and 20 cannot degrade the activity of the active ingredients of the composition. Examples of suitable vehicles include ointments, creams, gels, or suspensions, with or without purified collagen. The OPN protein, or fragments thereof, also may be impregnated into transdermal patches, plasters, and bandages, preferably in liquid or semi-liquid form.

25 The amount and/or biological activity of OPN in an animal can be increased, for example, by delivery of nucleic acid molecules encoding OPN, or a biologically active fragment thereof, into the body of an animal. By way of example, a vector which includes a nucleic acid molecule (typically a DNA molecule) that encodes an OPN protein can be introduced into any suitable host cell, including animal and 30 human cells, and the encoded OPN protein expressed therein. The vector can be

introduced into host cells *in vitro*, and the modified cells introduced into the body of an animal, or the vector can be introduced into cells, *in vivo*, within the body of an animal. Any art-recognized gene delivery method can be used to introduce a vector into one or more cells for expression therein, including: transduction, transfection,  
5 transformation, direct injection, electroporation, virus-mediated gene delivery, amino acid-mediated gene delivery, biolistic gene delivery, lipofection and heat shock. See, generally, Sambrook et al, *supra*. Representative, non-viral, methods of gene delivery into cells are disclosed in Huang, L., Hung, M-C, and Wagner, E., Non-Viral Vectors for Gene Therapy, Academic Press, San Diego, California (1999).

10 Expression vectors useful for expressing OPN protein, or biologically active fragments thereof, include chromosomal, episomal, and virus-derived vectors, e.g., vectors derived from bacterial plasmids, bacteriophages, yeast episomes, yeast chromosomal elements, viruses such as baculoviruses, papova viruses, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and  
15 vectors derived from combinations thereof, such as cosmids and phagemids. In certain embodiments in this regard, the vectors provide for specific expression, which may be inducible and/or cell type-specific. Among such expression vectors are those inducible by environmental factors that are easy to manipulate, such as temperature and nutrient additives.

20 For example, a coding sequence for OPN, or a biologically active fragment thereof, can be introduced into cells *in situ*, or after removal of the cells from the body, by means of viral vectors. For example, retroviruses are RNA viruses that have the ability to insert their genes into host cell chromosomes after infection. Retroviral vectors have been developed that lack the genes encoding viral proteins,  
25 but retain the ability to infect cells and insert their genes into the chromosomes of the target cell (A.D. Miller, *Hum. Gen. Ther.* 1:5-14 (1990)). Adenoviral vectors are designed to be administered directly to patients. Unlike retroviral vectors, adenoviral vectors do not integrate into the chromosome of the host cell. Instead, genes introduced into cells using adenoviral vectors are maintained in the nucleus as an  
30 extrachromosomal element (episome) that persists for a limited time period.

Adenoviral vectors will infect dividing and non-dividing cells in many different tissues *in vivo* including airway epithelial cells, endothelial cells, hepatocytes and various tumors (B.C. Trapnell, *Adv Drug Del Rev.* **12**:185-199 (1993)).

Another viral vector is the herpes simplex virus; a large, double-stranded  
5 DNA virus. Recombinant forms of the vaccinia virus can accommodate large inserts and are generated by homologous recombination. To date, this vector has been used to deliver, for example, interleukins (ILs), such as human IL-1 $\beta$  and the costimulatory molecules B7-1 and B7-2 (G.R. Peplinski et al., *Ann. Surg. Oncol.* **2**:151-9 (1995); J.W. Hodge et al., *Cancer Res.* **54**:5552-55 (1994)).

10 A plasmid vector can be introduced into mammalian cells in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid (e.g., LIPOFECTAMINE™; Life Technologies, Inc.; Rockville, Md.) or in a complex with a virus (such as an adenovirus) or components of a virus (such as viral capsid peptides). If the vector is a virus, it may be packaged in vitro using an appropriate  
15 packaging cell line and then transduced into host cells.

For example, a vector may be formulated for delivery either encapsulated in a lipid particle, a liposome, a vesicle, or a gene activated collagen matrix. Liposomes are vesicular structures characterized by a phospholipid bilayer membrane and an inner aqueous medium. Multilamellar liposomes have multiple lipid layers separated  
20 by aqueous medium. They form spontaneously when phospholipids are suspended in an excess of aqueous solution. The lipid components undergo self-rearrangement before the formation of closed structures and entrap water and dissolved solutes between the lipid bilayers.

Recently, liposomes were developed with improved serum stability and  
25 circulation half-times (see, e.g., U.S. Pat. No. 5,741,516). Furthermore, various methods of liposome and liposome-like preparations as potential drug carriers have been reviewed (see, e.g., U.S. Pat. No. 5,567,434; U.S. Pat. No. 5,552,157; U.S. Pat. No. 5,565,213; U.S. Pat. No. 5,738,868 and U.S. Pat. No. 5,795,587).

Additionally, studies have demonstrated that intramuscular injection of  
30 plasmid DNA formulated with 5% PVP (50,000 kDa) increases the level of reporter



gene expression in muscle as much as 200-fold over the levels found with injection of DNA in saline alone (R.J. Mumper et al., *Pharm. Res.* **13**:701-709 (1996); R.J. Mumper et al., *Proc. Intern. Symp. Cont. Rol. Bioac. Mater.* **22**:325-326 (1995)). Intramuscular administration of plasmid DNA results in gene expression that lasts for  
5 many months (J.A. Wolff et al., *Hum. Mol. Genet.* **1**:363-369 (1992); M. Manthorpe et al., *Hum. Gene Ther.* **4**:419-431 (1993); G. Ascadi et al., *New Biol.* **3**:71-81 (1991), D. Gal et al., *Lab. Invest.* **68**:18-25 (1993)).

Various devices have been developed for enhancing the availability of DNA to a target cell. A simple approach is to contact the target cell physically with  
10 catheters or implantable materials containing DNA (G.D. Chapman et al., *Circulation Res.* **71**:27-33 (1992)). Another method for achieving gene transfer involves using a fibrous collagen implant material soaked in a solution of DNA shortly before being placed in the site in which one desires to achieve gene transfer. The matrix may become impregnated with a gene DNA segment simply by soaking  
15 the matrix in a solution containing the DNA, such as a plasmid solution.

Another approach is to utilize needle-free, jet injection devices which project a column of liquid directly into the target tissue under high pressure. (P.A. Furth et al., *Anal. Biochem.* **20**:365-368 (1992); (H.L. Vahlsing et al., *J. Immunol. Meth.* **175**:11-22 (1994); (F.D. Ledley et al., *Cell Biochem.* **18A**:226 (1994)).

20 Another device for gene delivery is the "gene gun" or Biolistic™, a ballistic device that projects DNA-coated micro-particles directly into the nucleus of cells *in vivo*. Once within the nucleus, the DNA dissolves from the gold or tungsten microparticle and can be expressed by the target cell. This method has been used effectively to transfer genes directly into the skin, liver and muscle (N.S. Yang et al.,  
25 *Proc. Natl. Acad. Sci.* **87**:9568-9572 (1990); L. Cheng et al., *Proc. Natl. Acad. Sci. USA.* **90**:4455-4459 (1993); R.S. Williams et al., *Proc. Natl. Acad. Sci.* **88**:2726-2730 (1991)).

OPN proteins, or fragments thereof, may be immobilized onto (or within) a surface of an implantable or attachable medical device. The modified surface will  
30 typically be in contact with living tissue after implantation into an animal body. By

"implantable or attachable medical device" is intended any device that is implanted into, or attached to, tissue of an animal body, during the normal operation of the device (e.g., implantable drug delivery devices). Such implantable or attachable medical devices can be made from, for example, nitrocellulose, diazocellulose, glass, polystyrene, polyvinylchloride, polypropylene, polyethylene, dextran, Sepharose, agar, starch, and nylon. Linkage of the protein to a device can be accomplished by any technique that does not destroy the biological activity of the linked protein, for example by attaching one or both ends of the protein to the device. Attachment may also be made at one or more internal sites in the protein. Multiple attachments (both internal and at the ends of the protein) may also be used. A surface of an implantable or attachable medical device can be modified to include functional groups (e.g., carboxyl, amide, amino, ether, hydroxyl, cyano, nitrido, sulfanamido, acetylinic, epoxide, silanic, anhydric, succinimic, azido) for protein immobilization thereto. Coupling chemistries include, but are not limited to, the formation of esters, ethers, amides, azido and sulfanamido derivatives, cyanate and other linkages to the functional groups available on OPN proteins or fragments. OPN protein, or fragments thereof, can also be attached non-covalently by the addition of an affinity tag sequence to the protein, such as GST (Smith, D. B., and Johnson, K. S., *Gene* 67:31 (1988)), polyhistidines (Hochuli, E., et al., *J. Chromatog.* 411:77 (1987)), or biotin. Such affinity tags may be used for the reversible attachment of the protein to a device. The medical devices of the invention described herein can be used to deliver OPN proteins, or fragments thereof, to an animal body.

Methods of delivery of OPN proteins, or fragments thereof, also include administration by oral, pulmonary, parenteral (e.g., intramuscular, intraperitoneal, intravenous (IV) or subcutaneous injection), inhalation (such as via a fine powder formulation), transdermal, nasal, vaginal, rectal, or sublingual routes of administration, and can be formulated in dosage forms appropriate for each route of administration.

In another embodiment of the methods of the present invention, the amount and/or biological activity of thrombospondin 2 (TSP2) is decreased in an animal by a

method comprising the step of introducing into the animal an amount of a TSP2 antagonist effective to decrease the amount and/or biological activity of TSP2 in the animal. In the practice of this aspect of the invention, representative TSP2 antagonists include: TSP2 antisense nucleic acid molecules (such as antisense mRNA, antisense DNA or antisense oligonucleotides), TSP2 ribozymes, and molecules that inhibit the biological activity of TSP2 (such as anti-TSP2 antibodies, or a blocking peptide which interacts with TSP2 or a TSP2 receptor), thereby preventing TSP2 from eliciting a biological response. The methods of this aspect of the invention can be used to improve the wound response in an animal, and/or reduce the foreign body response in an animal.

An antisense nucleic acid molecule may be constructed in a number of different ways provided that it is capable of interfering with the expression of a target gene. For example, an antisense nucleic acid molecule can be constructed by inverting the coding region (or a portion thereof) of TSP2 relative to its normal orientation for transcription to allow the transcription of its complement.

The antisense nucleic acid molecule is usually substantially identical to at least a portion of the target gene or genes. The nucleic acid, however, need not be perfectly identical to inhibit expression. Generally, higher homology can be used to compensate for the use of a shorter antisense nucleic acid molecule. The minimal percent identity is typically greater than about 65%, but a higher percent identity may exert a more effective repression of expression of the endogenous sequence. Substantially greater percent identity of more than about 80% typically is preferred, though about 95% to absolute identity is typically most preferred.

The antisense nucleic acid molecule need not have the same intron or exon pattern as the target gene, and non-coding segments of the target gene may be equally effective in achieving antisense suppression of target gene expression as coding segments. A DNA sequence of at least about 30 or 40 nucleotides may be used as the antisense nucleic acid molecule, although a longer sequence is preferable. In the present invention, a representative example of a useful antagonist of TSP2 is an antisense TSP2 nucleic acid molecule which is at least ninety percent identical to the

complement of the TSP2 cDNA consisting of the nucleic acid sequence set forth in SEQ ID NO: 3. The nucleic acid sequence set forth in SEQ ID NO: 3 encodes the TSP2 protein consisting of the amino acid sequence set forth in SEQ ID NO: 4.

5 The targeting of antisense oligonucleotides to bind TSP2 mRNA is another mechanism that may be used to reduce the level of TSP2 protein synthesis. For example, the synthesis of polygalacturonase and the muscarine type 2 acetylcholine receptor are inhibited by antisense oligonucleotides directed to their respective mRNA sequences (U.S. Pat. No. 5,739,119 and U.S. Pat. No. 5,759,829). Furthermore, examples of antisense inhibition have been demonstrated with the  
10 nuclear protein cyclin, the multiple drug resistance gene (MDG1), ICAM-1, E-selectin, STK-1, striatal GABA<sub>A</sub> receptor and human EGF (*see, e.g.*, U.S. Pat. No. 5,801,154; U.S. Pat. No. 5,789,573; U.S. Pat. No. 5,718,709 and U.S. Pat. No. 5,610,288).

Ribozymes can also be utilized to decrease the amount and/or biological  
15 activity of TSP2, such as ribozymes which target TSP2 mRNA. Ribozymes are catalytic RNA molecules that can cleave nucleic acid molecules having a sequence that is completely or partially homologous to the sequence of the ribozyme. It is possible to design ribozyme transgenes that encode RNA ribozymes that specifically pair with a target RNA and cleave the phosphodiester backbone at a specific location,  
20 thereby functionally inactivating the target RNA. In carrying out this cleavage, the ribozyme is not itself altered, and is thus capable of recycling and cleaving other molecules. The inclusion of ribozyme sequences within antisense RNAs confers RNA-cleaving activity upon them, thereby increasing the activity of the antisense constructs.

25 Ribozymes useful in the practice of the invention typically comprise a hybridizing region, of at least about nine nucleotides, which is complementary in nucleotide sequence to at least part of the target TSP2 mRNA, and a catalytic region which is adapted to cleave the target TSP2 mRNA (*see generally*, EPA No. 0 321 201; WO88/04300; Haseloff & Gerlach, *Nature* 334:585-591 [1988]; Fedor &

Uhlenbeck, *Proc. Natl. Acad. Sci.: USA* 87:1668-1672 [1990]; Cech & Bass, *Ann. Rev. Biochem.* 55:599-629 [1986]).

Representative methods of delivery for antisense TSP2 molecules, and/or TSP2 ribozymes, include any of the methods of delivering nucleic acid molecules  
5 into living cells described in this patent application.

In another embodiment of this aspect of the present invention, the TSP2 antagonist is an anti-TSP2 antibody. By way of representative example, antigen useful for raising antibodies can be prepared in the following manner. A nucleic acid molecule (such as a TSP2 cDNA molecule) is cloned into a plasmid vector, such as a  
10 Bluescript plasmid (available from Stratagene, Inc., La Jolla, California). The recombinant vector is then introduced into an *E. coli* strain (such as *E. coli* XL1-Blue, also available from Stratagene, Inc.) and the polypeptide encoded by the nucleic acid molecule is expressed in *E. coli* and then purified. Alternatively, polypeptides can be prepared using peptide synthesis methods that are well known in  
15 the art. The synthetic polypeptides can then be used to prepare antibodies. Direct peptide synthesis using solid-phase techniques (Stewart et al., *Solid-Phase Peptide Synthesis*, W H Freeman Co, San Francisco Calif. (1969); Merrifield, *J. Am. Chem. Soc.* 85:2149-2154 (1963) is an alternative to recombinant or chimeric peptide production. Automated synthesis may be achieved, for example, using Applied  
20 Biosystems 431A Peptide Synthesizer (Foster City, Calif.) in accordance with the instructions provided by the manufacturer. Methods for preparing monoclonal and polyclonal antibodies are well known to those of ordinary skill in the art and are set forth, for example, in chapters five and six of *Antibodies A Laboratory Manual*, E. Harlow and D. Lane, Cold Spring Harbor Laboratory (1988). Antibody production  
25 includes not only the stimulation of an immune response by injection into animals, but also analogous processes such as the production of synthetic antibodies, the screening of recombinant immunoglobulin libraries for specific-binding molecules (Orlandi et al., *Proc. Natl. Acad. Sci. USA* 86:3833, 1989, or Huse et al. *Science* 256:1275, 1989), or the *in vitro* stimulation of lymphocyte populations.

The invention also extends to non-antibody polypeptides, sometimes referred to as blocking peptides, that have been designed to bind specifically to, and inhibit the active site of, TSP2, or a TSP2 binding partner, or a receptor of TSP2. For example, the domain of TSP2 which binds to the receptor CD36 can be targeted with a blocking peptide. Other examples of the design of such peptides, which possess a prescribed ligand specificity are given in Beste et al. (1999, Proceedings of the National Academy of Science 96:1898-1903).

An additional strategy suitable for suppression of target gene activity entails the sense expression of a mutated or partially deleted form of the protein encoded by the target gene according to general criteria for the production of dominant negative mutations (Herskowitz I, *Nature* 329: 219-222 (1987)).

Representative methods of delivery for anti-TSP2 antibodies and/or blocking peptides include any of the protein delivery methods disclosed in this patent application.

In another aspect, the present invention provides medical devices comprising (a) a device body; and (b) a surface layer attached to the device body, the surface layer including an amount of an agonist or antagonist of a matricellular protein sufficient to reduce the foreign body response against the medical device, wherein the device is adapted to be affixed to, or implanted within, the soft tissue of an animal.

Some medical devices of the invention are adapted to be implanted into the soft tissue of an animal, such as a mammal, including a human, during the normal operation of the medical device. Implantable medical devices of the invention may be completely implanted into the soft tissue of an animal body (*i.e.*, the entire device is implanted within the body), or the device may be partially implanted into an animal body (*i.e.*, only part of the device is implanted within an animal body, the remainder of the device being located outside of the animal body). Representative examples of completely implantable medical devices include, but are not limited to: cardiovascular devices (such as vascular grafts and stents), artificial blood vessels, artificial bone joints, such as hip joints, and scaffolds that support tissue growth (in

such anatomical structures as nerves, pancreas, eye and muscle). Representative examples of partially implantable medical devices include: biosensors (such as those used to monitor the level of drugs within a living body, or the level of blood glucose in a diabetic patient) and percutaneous devices (such as catheters) that penetrate the skin and link a living body to a medical device, such as a kidney dialysis machine.

Some medical devices of the invention are adapted to be affixed to soft tissue of an animal, such as a mammal, including a human, during the normal operation of the medical device. These medical devices are typically affixed to the skin of an animal body. Examples of medical devices that are adapted to be affixed to soft tissue of an animal include skin substitutes, and wound or burn treatment devices (such as surgical bandages, transdermal patches and hydrogels).

The device body can be made from any suitable material. Representative examples of synthetic polymers useful for making the device body include: (poly)urethane, (poly)carbonate, (poly)ethylene, (poly)propylene, (poly)lactic acid, (poly)galactic acid, (poly)acrylamide, (poly)methyl methacrylate and (poly)styrene. Useful natural polymers include collagen, hyaluronic acid and elastin.

The surface layer can cover the whole of the device body, or one or more parts of the device body, such as areas of the device body where it is desired to reduce the foreign body response. The surface layer can be made, for example, from any suitable material that: (a) permits deposition therein, or attachment thereto, of an amount of an agonist, or antagonist, of a matricellular protein sufficient to reduce the foreign body response against the medical device; and (b) can be attached to the device body (before or after deposition within, or attachment to, the surface layer of an amount of an agonist, or antagonist, of a matricellular protein sufficient to reduce the foreign body response against the medical device). Representative examples of materials useful for making the surface layer include porous matrices. Porous matrices are useful, for example, for delivering antisense TSP2 molecules to an animal body.

Representative porous matrices useful for making the surface layer are those prepared from tendon or dermal collagen, as may be obtained from a variety of

commercial sources, (e.g., Sigma and Collagen Corporation), or collagen matrices prepared as described in U.S. Pat. Nos. 4,394,370 and 4,975,527. One collagenous material is termed UltraFiber™, and is obtainable from Norian Corp. (Mountain View, Calif.).

5           Certain polymeric matrices may also be employed if desired, these include acrylic ester polymers and lactic acid polymers, as disclosed, for example, in U.S. Pat. Nos. 4,526,909, and 4,563,489. Particular examples of useful polymers are those of orthoesters, anhydrides, propylene-cofumarates, or a polymer of one or more  $\alpha$ -hydroxy carboxylic acid monomers, (e.g.  $\alpha$ -hydroxy acetic acid (glycolic acid)  
10           and/or  $\alpha$ -hydroxy propionic acid (lactic acid).

          The surface layer can be made, for example, by attachment of matricellular protein(s) to the device body, for example by covalent activation of the surface of the medical device. By way of representative example, matricellular protein(s) can be attached to the device body by any of the following pairs of reactive groups (one  
15           member of the pair being present on the surface of the device body, and the other member of the pair being present on the matricellular protein(s): hydroxyl/carboxylic acid to yield an ester linkage; hydroxyl/anhydride to yield an ester linkage; hydroxyl/isocyanate to yield a urethane linkage.

          A surface of a device body that does not possess useful reactive groups can be  
20           treated with radio-frequency discharge plasma (RFGD) etching to generate reactive groups in order to allow deposition of matricellular protein(s) (e.g., treatment with oxygen plasma to introduce oxygen-containing groups; treatment with propyl amino plasma to introduce amine groups). When an RFGD glow discharge plasma is created using an organic vapor, deposition of a polymeric overlayer occurs on the  
25           exposed surface. RFGD plasma deposited films offer several unique advantages. They are smooth, conformal, and uniform. Film thickness is easily controlled and ultrathin films (10-1000 Angstroms) are readily achieved, allowing for surface modification of a material without alteration to its bulk properties. Moreover, plasma films are highly-crosslinked and pin-hole free, and therefore chemically stable and  
30           mechanically durable. RFGD plasma deposition of organic thin films has been used



in microelectronic fabrication, adhesion promotion, corrosion protection, permeation control, as well as biomaterials. (see, e.g., Ratner, US Pat No 6,131,580).

5 An amount of an agonist or antagonist of a matricellular protein sufficient to reduce the foreign body response to the implanted medical device is included in or on a surface layer of the medical device. Agonists or antagonists of a matricellular protein include, for example: proteins, peptides, antibodies, and nucleic acid molecules. Useful, representative, examples of TSP2 antagonists include: TSP2 antisense nucleic acid molecules (such as antisense mRNA, antisense DNA or antisense oligonucleotides), TSP2 ribozymes, and molecules that inhibit the biological activity of TSP2 (such as anti-TSP2 antibodies, or a blocking peptide which interacts with TSP2 or a TSP2 receptor), thereby preventing TSP2 from eliciting a biological response. OPN, or OPN fragments retaining the ability to reduce the foreign body response, can be included in the surface layer of the medical device. Any combination of agonists and/or antagonists of a matricellular protein can be included in or on a surface layer of a medical device of the invention.

FIGURE 1 shows a representative medical device 10 of the present invention, in the form of an implantable drug delivery device, which includes a device body 12 to which is attached a surface layer 14. In the embodiment shown in FIGURE 1, surface layer 14 has been partially removed to show device body 12 beneath. Device body 12 is indicated by hatching. As shown in the cross-sectional view of medical device 10 in FIGURE 2, surface layer 14 includes a surface layer body 16 that defines an internal surface 18, attached to device body 12, and an external surface 20.

In the representative embodiment of device 10 shown in FIGURES 1 and 2, surface layer 14 is made from a porous matrix. FIGURE 3 shows a representation of porous matrix 22 within which are disposed molecules 24 of an agonist or antagonist of a matricellular protein (other molecules, such as drugs, may also be disposed within porous matrix 22). Thus, in operation, device 10 is implanted into the soft tissue of an animal body where molecules 24 are released over time and reduce the foreign body response by the animal body against implanted device 10.

Some medical devices 10 of the invention include a multiplicity of surface layers 14 disposed one upon the other, wherein at least one of surface layers 14 includes an agonist or antagonist of a matricellular protein. A "multiplicity" is defined as at least two surface layers 14, and each surface layer 14 may be made from the same material as the other surface layer(s) 14, or from a different material. By way of representative example, FIGURE 4 shows a medical device 10 of the invention, in the form of an implantable drug delivery device, that includes a first surface layer 14' disposed upon a second surface layer 14". First surface layer 14' includes molecules of osteopontin 26 disposed therein. Second surface layer 14" includes molecules of a thrombospondin 2 antagonist 28 disposed therein. First surface layer 14' is located externally to second surface layer 14" in that first surface layer 14' is located further from device body 12 than second surface layer 14", and first surface layer 14' defines an external surface 30 of medical device 10. Thus, when implanted into an animal body, the embodiment of medical device 10 shown in FIGURE 4 first releases osteopontin 26 into the surrounding tissue, then releases thrombospondin 2 antagonist 28 into the surrounding tissue.

FIGURE 5 shows a representative embodiment of a medical device 10 of the invention, in the form of a drug delivery device, that includes a device body 12 and a surface layer 14 disposed on device body 12. Surface layer 14 includes a first area 32, including a first agonist or first antagonist of a matricellular protein, and a second area 34, including a second agonist or second antagonist of a matricellular protein. The first agonist is different from the second agonist, and the first antagonist is different from the second antagonist. Thus, for example, first area 32 can include osteopontin protein, or nucleic acid molecules encoding osteopontin, and second area 34 can include a TSP2 antagonist 28, such as a TSP2 antisense nucleic acid molecule, an immobilized anti-TSP2 antibody, or an anti-TSP2 blocking peptide.

One of ordinary skill in the art will appreciate that surface layers 14 can be configured and arranged to optimize the timing of the delivery of one or more agonists and/or antagonists of a matricellular protein in order to reduce the foreign body response. For example, typically antisense TSP2 molecules are not fixedly

attached to, or within, surface layer 14 so that the antisense TSP2 molecules are free to diffuse out of surface layer 14 and be taken up by the cells of surrounding tissue. Typically, however, osteopontin protein is fixedly attached, such as by covalent linkage, to, or within, surface layer 14 to prevent movement of the protein away from the wound site. It is understood by one of ordinary skill in the art that any combination of agonist and/or antagonist of one or more matricellular proteins may be included in surface layer 14.

The following examples merely illustrate the best mode now contemplated for practicing the invention, but should not be construed to limit the invention. All literature citations herein are expressly incorporated by reference.

#### Example 1

This example describes the increase in blood vessel density that occurs within a foreign body capsule as a result of the presence of a TSP2 antisense cDNA molecule in the surface layer of an implanted device.

Construction of plasmids: Sense and antisense TSP2 expression plasmids were generated by ligation of a 3.5-kb EcoR1 fragment of mouse TSP2 (mTSP2) cDNA into the mammalian expression vector pZeoSV (Invitrogen, San Diego, CA). The size and orientation of inserts were confirmed by restriction digestion with Xho1.

Generation of TSP2-null mice: These mice were generated as described (Kyriakides et al., 1998, J. Cell Biol. 140: 419-430).

Preparation of devices: The devices were each made from a millipore filter coated with a collagen matrix. The collagen matrices were impregnated with a plasmid including either a TSP2 sense, or TSP2 antisense, nucleic acid molecule. Some collagen matrices were not impregnated with a plasmid. Equal amounts (1 mg) of neutralized collagen and plasmid DNA were mixed at 4°C. Implants were bathed in this solution, placed at -70° C and then lyophilized to generate a dry gene activated matrix.

Implantation of devices: the devices were implanted into TSP2-null mice, and into control mice, for a 2-4 week period.

Measurement of capsule neovascularization: At 2-4 weeks post implant, the number of blood vessels per visual field was measured in the capsules surrounding the implant. Histological sections were stained with antibodies to PECAM-1 and visualized with the peroxidase reaction. The number and size of blood vessels was  
5 determined from microscopic digital images collected at 400x magnification and analyzed by imaging software.

As shown in FIGURE 6, foreign body capsules formed around uncoated filters implanted into TSP2-null animals displayed an increase in blood vessel density as compared to the wild type animals treated similarly. This demonstrates that in the  
10 absence of TSP2, there is an increase in neovascularization of the foreign body capsule surrounding an implant.

Under conditions designed to test TSP2 complementation, TSP2 null animals were implanted with devices comprising a millipore filter coated with a collagen matrix impregnated with a plasmid including a TSP2 cDNA in sense orientation. As  
15 shown in FIGURE 6, the addition of the sense TSP2 construct led to a reduction in the vessel density within the foreign body capsule, similar to that seen in the wild-type mice, while the antisense TSP2 construct did not change the vessel density in the TSP2 null mice.

Wild-type animals implanted with a device including a surface collagen layer  
20 including an antisense TSP2 construct displayed an increase in foreign body capsule blood vessel density, while no change was observed in wild-type animals implanted with a device including a sense TSP2 construct. These results were especially significant in light of the overall reduction in vascularity observed in controls in which the collagen matrix alone was coated onto the millipore filters.

25 The results above suggest that *in vivo* delivery of TSP2 antisense cDNA via a medical device of the invention can eliminate the anti-angiogenic activity of TSP2 and thereby promote vascularization of the foreign body capsule surrounding an implanted medical device.

## Example 2

This example shows that OPN-null mice demonstrate high levels of foreign body giant cells surrounding an implant as compared to wild type mice.

One of the hallmarks of the foreign body response is the appearance of foreign body giant cells or macrophages that have fused together as a result of encountering an implanted foreign material. As many as one hundred cells fuse to form a syncytium containing as many as one hundred nuclei. In order to address the role of OPN in the foreign body response, OPN null mice (knockout mice) and normal control mice were implanted with fixed bovine pericardium and analyzed at 14 days and 30 days post implant for the appearance of foreign body giant cells.

Generation of OPN null mice: The mice utilized in these experiments are described in Liaw, L. et al., J. Clin Invest, 1998 **101**(7):1468-78, which publication is incorporated herein by reference.

Preparation of bovine pericardium implant samples: Glutaraldehyde-fixed bovine pericardial tissues were a gift from Edwards Lifesciences. Bovine pericardial tissues were excised, fixed and stored in 0.6% glutaraldehyde, pH 7.0, until use.

Method of implantation: 4 mm<sup>2</sup> biopsy punches of glutaraldehyde-fixed aortic valve leaflets (GFAV) were prepared, washed extensively in sterile PBS, and subcutaneously implanted into the dorsal side of anaesthetized 5-6 week old, female OPN +/+ or -/- mice (two GFAV per mouse). At the indicated times, mice were euthanized, and implants removed for histological analysis. All protocols were approved by the animal use committee, University of Washington.

Foreign body giant cell formation: The OPN null mice and control mice were analyzed at 14 days and 30 days post implant for the appearance of foreign body giant cells. As shown in FIGURE 7, at both 14 days and 30 days post-implantation, the OPN null mice had higher levels of foreign body giant cells than the control mice. These results suggest that increasing the amount and/or biological activity of OPN will decrease the number of foreign body giant cells and thereby reduce the foreign body reaction to an implant.

This example shows that OPN immobilized in the surface layer of an implanted device causes a reduction in both fibrous capsule thickness and the amount of macrophage infiltration of the fibrous capsule surrounding the implanted device.

Preparation of polyethylene discs: some polyethylene discs were uncoated  
5 while others were coated with a non-fouling RFGD tetraglyme coating. Some tetraglyme-coated discs also included osteopontin that was covalently attached to the tetraglyme coating.

Tetraglyme coatings were prepared by subjecting the disks to Radio  
Frequency Plasma Discharge deposition of vapor phase tetra (ethylene) glycol  
10 dimethyl ether (tetra GLYME) as described in U.S. Patents 5,153,072, and 5,002,794, (both of which patents are incorporated herein by reference). The GLYME-coated disks were sterilized with 70% ethanol/water, and filter-sterilized solutions of osteopontin were covalently immobilized to the tetraglyme coating by  
15 using disuccimidyl carbonate to activate carboxyls and hydroxyls on the glyme surface, either by reacting an allylamine glyme film with succinic anhydride, or by using the native reactive groups of the glyme film.

Method of implanting polyethylene disks : Materials were implanted in at least quadruplicate (into 4 different mice) for four weeks. Strict aseptic technique were used. All materials were sterilized by an overnight soak in sterile 70% ethanol,  
20 followed by three 20-minute washes in sterile, pyrogen free water. All instruments were autoclaved prior to surgery, and soaked in 70% ethanol between animals.

The materials were surgically implanted beneath the skin on the backs (dorsal side) of male mice using aseptic technique. Animals were anesthetized with a cocktail of ketamine and xylazine. The incision site was prepared by shaving,  
25 swabbing with Betadine followed by a 70% alcohol wipe. A single 1-1.5 cm incision was made midline on the back of each mouse, and two subcutaneous pockets were created by blunt dissection lateral to each side of the incision. One implant was placed in each pocket, and the incision was closed with sterile wound clips. Occasionally a second incision was made to accommodate two more implants, using  
30 the exact procedure as described above. Animals were allowed to recover prior to

returning to housing cages. Animals were given food and water *ad libitum* for the remainder of the four week study.

After four weeks, animals were sacrificed by CO<sub>2</sub> asphyxiation, wound clips were removed, and implants were retrieved *en-bloc* in an effort to not disturb the biomaterial/host tissue interface. Explants were fixed with methyl Carnoy's or embedded and frozen immediately in liquid nitrogen. Chemically fixed explants were processed, embedded in paraffin and sectioned. Several sections of each explant were stained with haematoxylin and eosin (H&E) or Masson's trichrome. The remaining sections were kept in reserve for immunocytochemical staining.

Quantification of foreign body capsule thickness: Tissue samples were taken from wild-type mice at four weeks after implantation of polyethylene disks which were either uncoated, coated with a non-fouling (RFGD tetraglyme) coating, or coated with a non-fouling (RFGD tetraglyme) coating that included OPN covalently immobilized to the glyme coating. The thickness of the foreign body capsule surrounding the implants was measured. Capsule thickness was measured by light microscopy using an ocular reticule that had been previously calibrated using a stage micrometer. 5 equi-distant points along the length of a single section were chosen for measurement, and capsule thickness was measured at the tissue/material interface on both surfaces of the implant (skin side and fat or muscle side) at each of these 5 points. Thus, 10 measurements were made for each implant.

As shown in FIGURE 8A, the uncoated disk resulted in the thickest capsule, the glyme coating reduced the thickness of the foreign body capsule, and the glyme coating including the immobilized OPN was associated with a marked reduction in fibrous capsule thickness.

Quantification of macrophage infiltration: The tissue samples as described above were also analyzed with respect to macrophage infiltration of the foreign body capsule. As shown in FIGURE 8B, the results correlated with the capsule thickness; the uncoated disks had the highest level of macrophage infiltration, followed by the glyme coating, and the lowest macrophage score was found in the glyme coating containing the immobilized OPN.

These results demonstrate that OPN immobilized on the surface of a device implanted into an animal body reduces the foreign body reaction to the implant.

While the preferred embodiment of the invention has been illustrated and described, it will be appreciated that various changes can be made therein without  
5 departing from the spirit and scope of the invention.



The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A method of modulating the amount or biological activity of thrombospondin 2 or osteopontin in an animal, said method comprising the step of introducing into the animal an amount of a molecule, selected from the group consisting of osteopontin and a thrombospondin 2 antagonist, effective to modulate the amount or biological activity of thrombospondin 2 or osteopontin in the animal.

2. The method of Claim 1 wherein an antagonist of thrombospondin 2 is introduced into the animal.

3. The method of Claim 2 wherein the amount or biological activity of thrombospondin 2 is decreased by said antagonist of thrombospondin 2.

4. The method of Claim 2 wherein the thrombospondin 2 antagonist is selected from the group consisting of an antisense thrombospondin 2 nucleic acid molecule, an anti-thrombospondin 2 antibody, a thrombospondin 2 blocking peptide and a thrombospondin 2 ribozyme.

5. The method of Claim 4 wherein an antisense thrombospondin 2 nucleic acid molecule is introduced into the animal.

6. The method of Claim 5 wherein the antisense thrombospondin 2 nucleic acid molecule is at least ninety percent identical to the complement of a thrombospondin 2 cDNA consisting of the nucleic acid sequence set forth in SEQ ID NO. 3.

7. The method of Claim 5 wherein the antisense thrombospondin 2 nucleic acid molecule hybridizes under stringent conditions to a thrombospondin 2 cDNA molecule consisting of the nucleic acid sequence set forth in SEQ ID NO. 3.

8. The method of Claim 4 wherein an anti-thrombospondin 2 antibody is introduced into the animal.

9. The method of Claim 4 wherein a thrombospondin 2 blocking peptide is introduced into the animal.

10. The method of Claim 4 wherein a thrombospondin 2 ribozyme is introduced into the animal.

11. The method of Claim 1 wherein osteopontin is introduced into the animal.

12. The method of Claim 1 wherein the molecule is introduced into the animal by a method selected from the group consisting of injection, as a component of a lipid complex, as a component of an implanted porous matrix, and by immobilization onto an implanted surface.

13. The method of Claim 5 wherein an antisense thrombospondin 2 nucleic acid molecule is incorporated within a delivery device which is introduced into the animal.

14. The method of Claim 13 wherein the delivery device comprises a porous matrix wherein the thrombospondin 2 antisense nucleic acid molecule is disposed.

15. The method of Claim 1 wherein the animal is exhibiting a wound response, and the amount of the introduced molecule is effective to [reduce] improve the wound response.

16. The method of Claim 15 wherein the molecule is an antisense thrombospondin 2 nucleic acid molecule.

17. The method of Claim 1 wherein osteopontin and an antagonist of thrombospondin 2 are introduced into the animal.

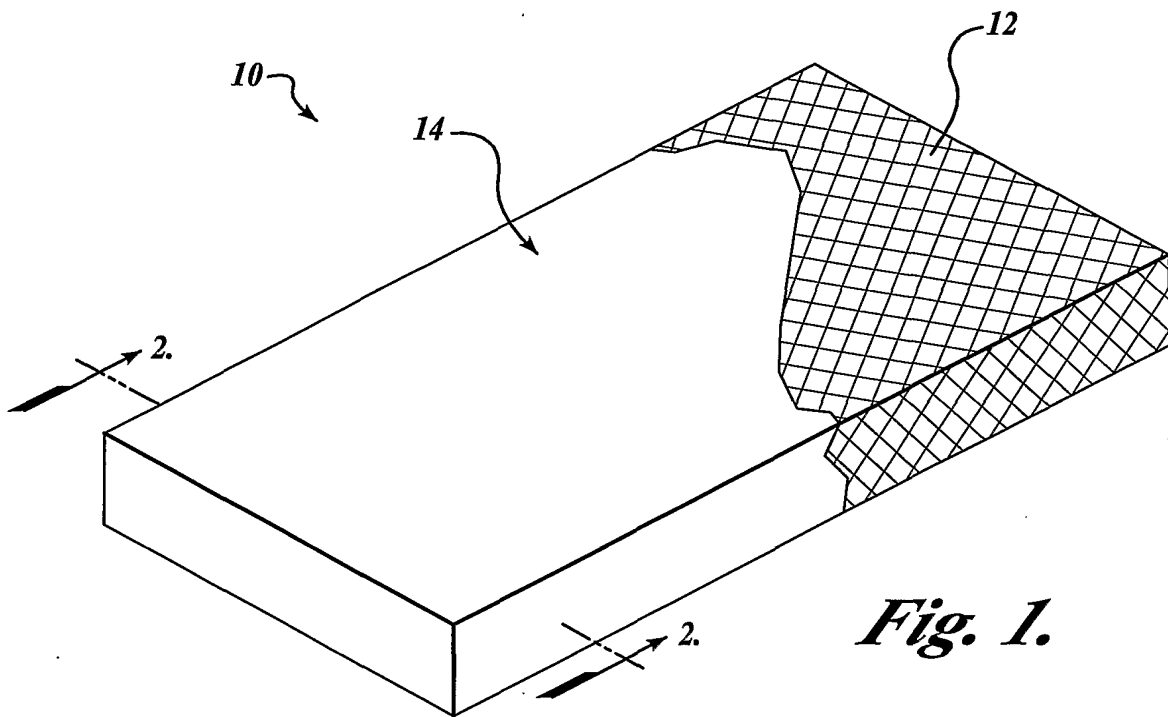
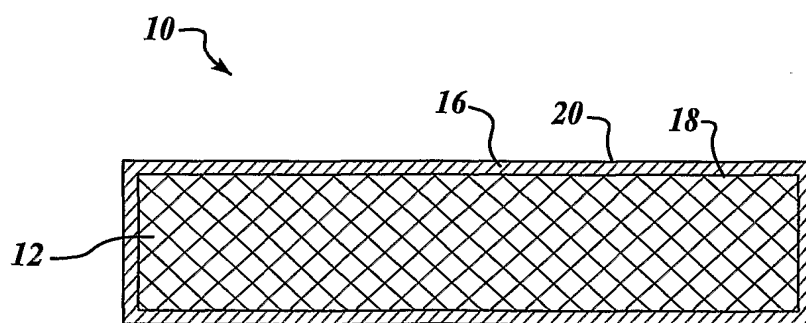
18. The method of Claim 17 wherein the antagonist to thrombospondin 2 is an antisense thrombospondin 2 nucleic acid molecule.

19. A medical device comprising:
- (a) a device body; and
  - (b) a surface layer attached to the device body, said surface layer comprising an amount of an agonist or antagonist of a matricellular protein sufficient to reduce the foreign body response against the device, wherein said device is adapted to be affixed to, or implanted within, the soft tissue of an animal.
20. The medical device of Claim 19 wherein the device is selected from the group of devices consisting of wholly implanted medical devices, partially implanted medical devices, and surface medical devices.
21. The medical device of Claim 19 wherein the surface layer attached to the device body comprises a porous matrix.
22. The medical device of Claim 19 further comprising a multiplicity of surface of layers disposed one upon the other, wherein at least one of said surface layers comprises an agonist or antagonist of a matricellular protein.
23. The medical device of Claim 22 wherein the device comprises: (a) a first surface layer comprising a first agonist, or first antagonist, of a matricellular protein; and (b) a second surface layer comprising a second agonist, or second antagonist, of a matricellular protein, wherein said first agonist is different from said second agonist and said first antagonist is different from said second antagonist.
24. The medical device of Claim 22 wherein the device comprises: (a) a first surface layer comprising osteopontin; and (b) a second surface layer comprising a thrombospondin 2 antagonist, wherein said first surface layer is disposed external to said second surface layer.
25. The medical device of Claim 24 wherein said thrombospondin 2 antagonist is an antisense nucleic acid molecule.

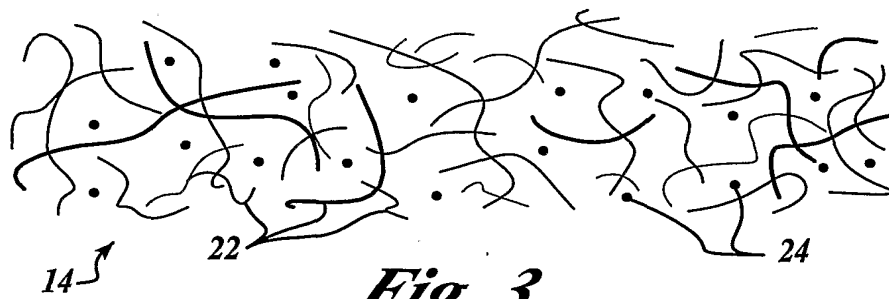
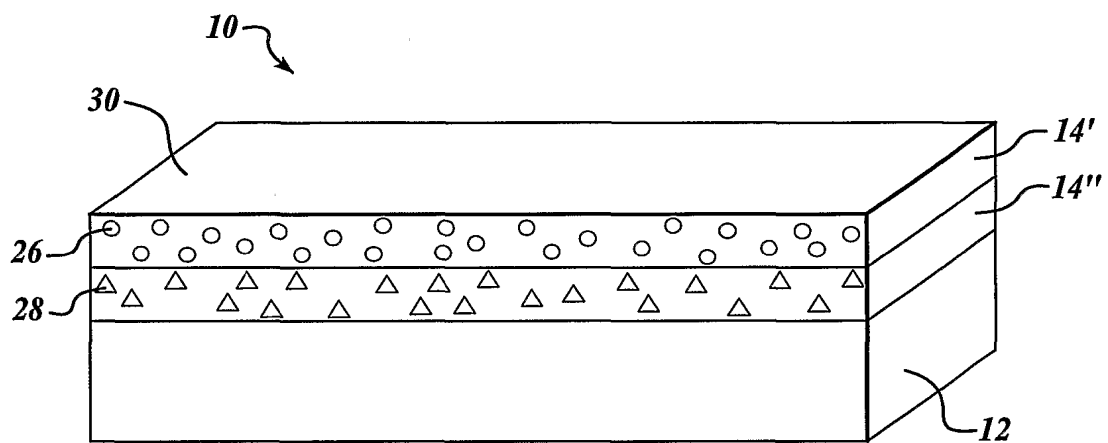
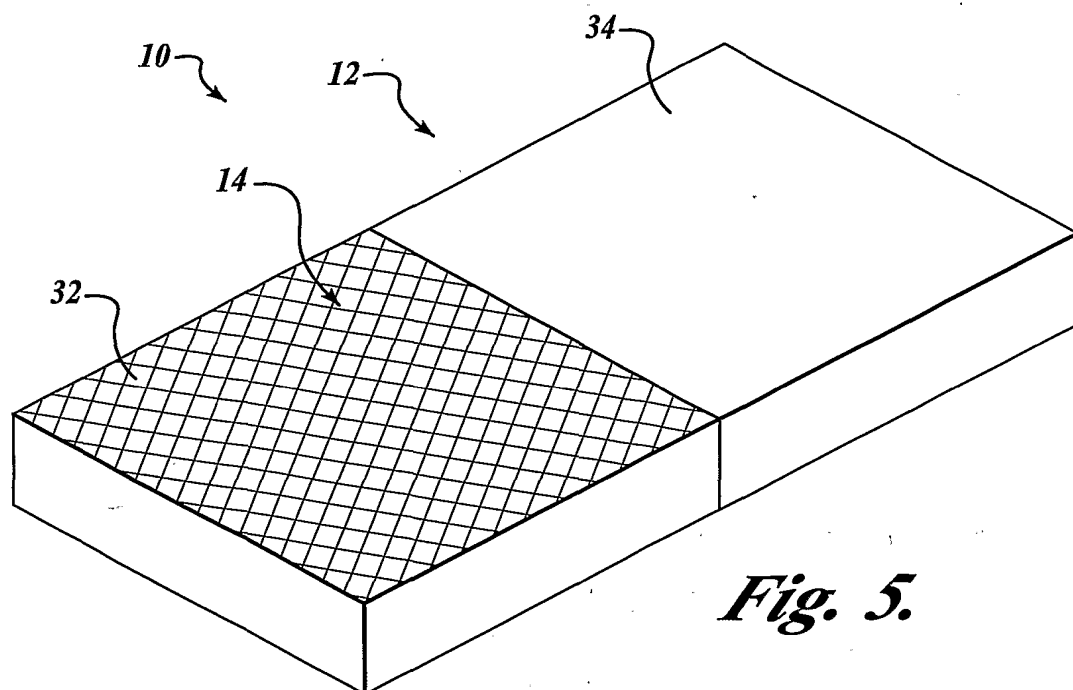
26. The medical device of Claim 19 wherein the surface layer comprises:  
(a) a first area comprising a first agonist or first antagonist of a matricellular protein;  
and (b) a second area comprising a second agonist or second antagonist, wherein the  
first agonist is different from the second agonist and the first antagonist is different  
from the second antagonist.

27. The method of Claim 1 wherein the animal is exhibiting a foreign  
body response, and the amount of the introduced molecule is effective to reduce the  
foreign body response.

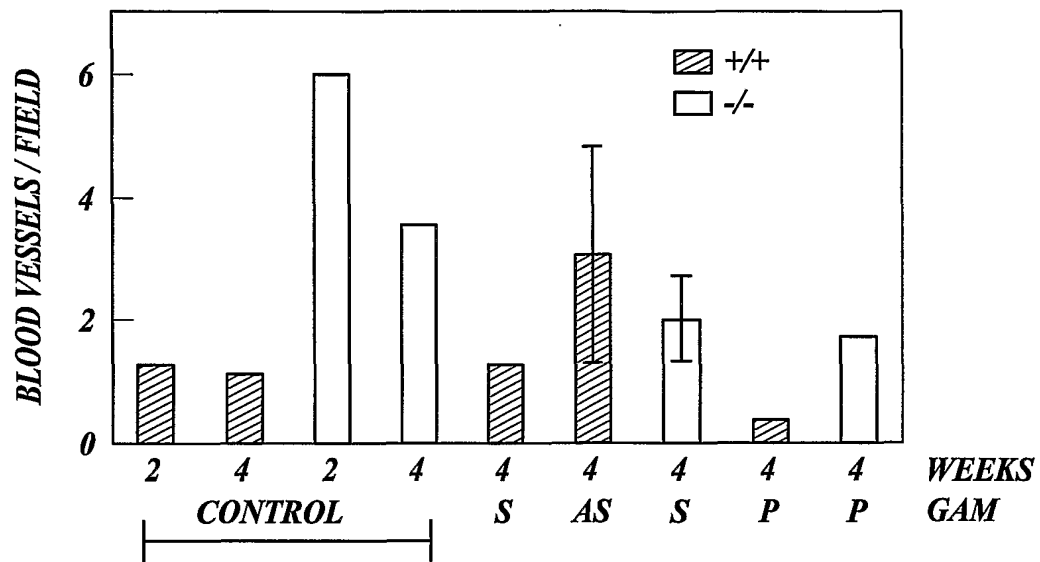
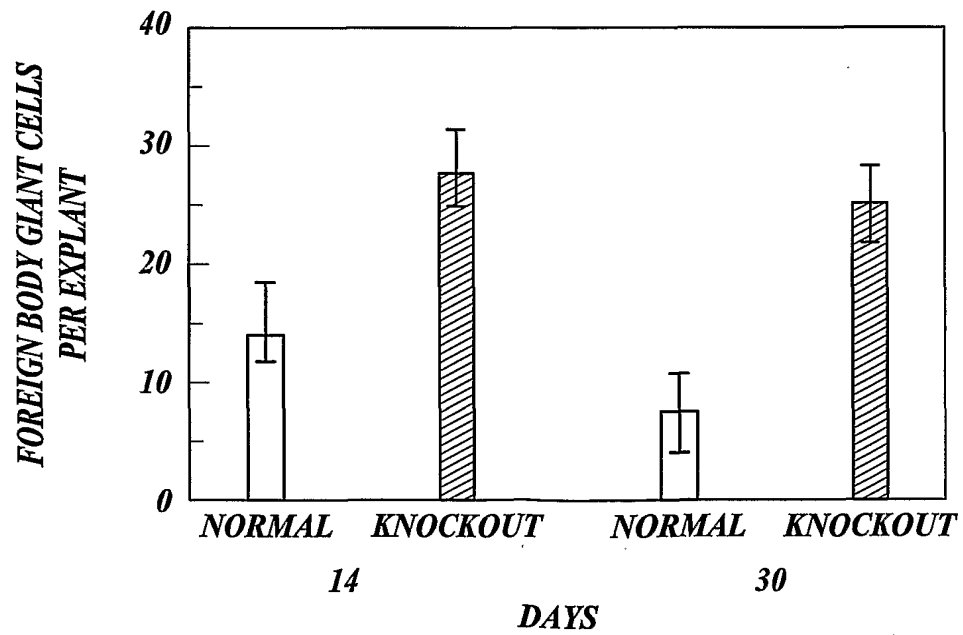
1/4

*Fig. 1.**Fig. 2.*

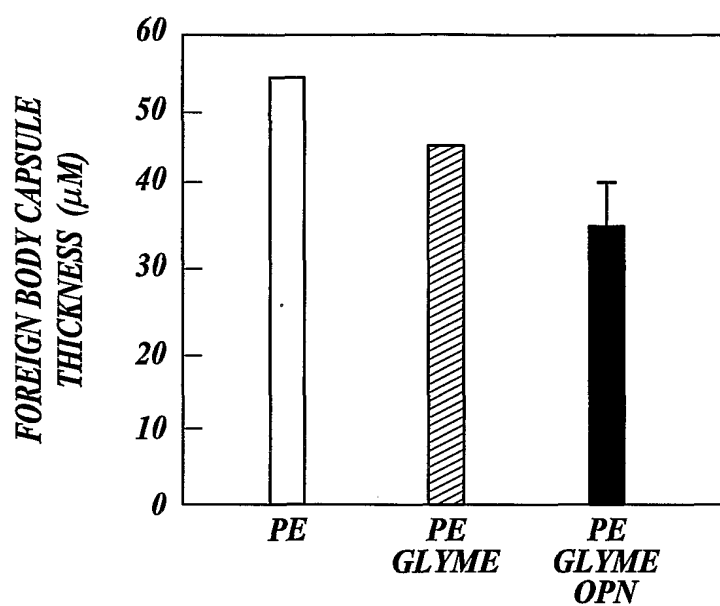
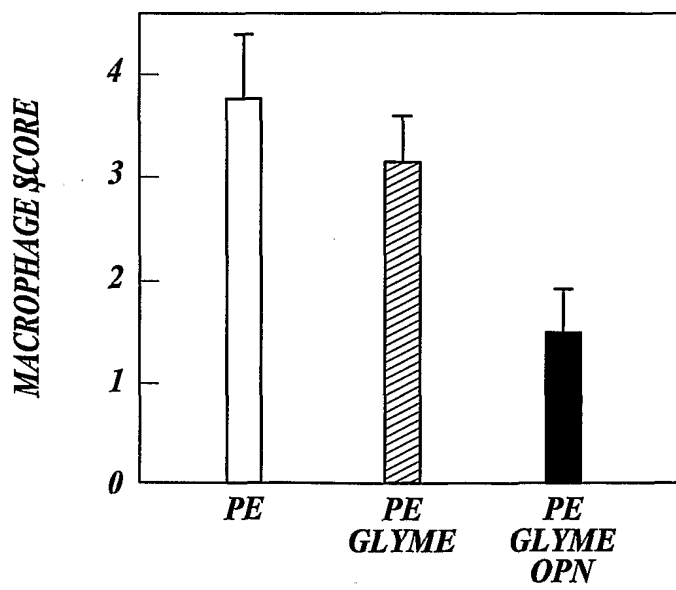
2/4

*Fig. 3.**Fig. 4.**Fig. 5.*

3/4

*Fig. 6.**Fig. 7.*

4/4

*Fig. 8A.**Fig. 8B.*



## SEQUENCE LISTING

&lt;110&gt; University of Washington

&lt;120&gt; Methods and Devices to Modulate the Wound Response

&lt;130&gt; UOFW117807

&lt;150&gt; US 60/222,071

&lt;151&gt; 2000-08-01

&lt;160&gt; 4

&lt;170&gt; PatentIn version 3.0

&lt;210&gt; 1

&lt;211&gt; 1469

&lt;212&gt; DNA

&lt;213&gt; Homo Sapien

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (102)..(1001)

&lt;400&gt; 1

```

agcagcagga ggaggcagag cacagcatcg tcgggaccag actcgtctca ggccagttgc      60
agcctttctca gccaaacgcc gaccaaggaa aactcactac c atg aga att gca gtg      116
                                         Met Arg Ile Ala Val
                                         1             5

att tgc ttt tgc ctc cta ggc atc acc tgt gcc ata cca gtt aaa cag      164
Ile Cys Phe Cys Leu Leu Gly Ile Thr Cys Ala Ile Pro Val Lys Gln
                        10                        15                        20

gct gat tct gga agt tct gag gaa aag cag ctt tac aac aaa tac cca      212
Ala Asp Ser Gly Ser Ser Glu Glu Lys Gln Leu Tyr Asn Lys Tyr Pro
                        25                        30                        35

gat gct gtg gcc aca tgg cta aac cct gac cca tct cag aag cag aat      260
Asp Ala Val Ala Thr Trp Leu Asn Pro Asp Pro Ser Gln Lys Gln Asn
                        40                        45                        50

ctc cta gcc cca cag acc ctt cca agt aag tcc aac gaa agc cat gac      308
Leu Leu Ala Pro Gln Thr Leu Pro Ser Lys Ser Asn Glu Ser His Asp
                        55                        60                        65

cac atg gat gat atg gat gat gaa gat gat gat gac cat gtg gac agc      356
His Met Asp Asp Met Asp Asp Glu Asp Asp Asp Asp His Val Asp Ser
70                        75                        80                        85

cag gac tcc att gac tcg aac gac tct gat gat gta gat gac act gat      404
Gln Asp Ser Ile Asp Ser Asn Asp Ser Asp Asp Val Asp Asp Thr Asp
                        90                        95                        100

gat tct cac cag tct gat gag tct cac cat tct gat gaa tct gat gaa      452
Asp Ser His Gln Ser Asp Glu Ser His His Ser Asp Glu Ser Asp Glu
                        105                        110                        115

ctg gtc act gat ttt ccc acg gac ctg cca gca acc gaa gtt ttc act      500
Leu Val Thr Asp Phe Pro Thr Asp Leu Pro Ala Thr Glu Val Phe Thr
                        120                        125                        130

cca gtt gtc ccc aca gta gac aca tat g t ggc cga ggt gat agt gtg      548

```

Pro	Val	Val	Pro	Thr	Val	Asp	Thr	Tyr	Asp	Gly	Arg	Gly	Asp	Ser	Val		
135						140					145						
gtt	tat	gga	ctg	agg	tca	aaa	tct	aag	aag	ttt	cgc	aga	cct	gac	atc		596
Val	Tyr	Gly	Leu	Arg	Ser	Lys	Ser	Lys	Lys	Phe	Arg	Arg	Pro	Asp	Ile		
150					155					160					165		
cag	tac	cct	gat	gct	aca	gac	gag	gac	atc	acc	tca	cac	atg	gaa	agc		644
Gln	Tyr	Pro	Asp	Ala	Thr	Asp	Glu	Asp	Ile	Thr	Ser	His	Met	Glu	Ser		
				170					175					180			
gag	gag	ttg	aat	ggc	gca	tac	aag	gcc	atc	ccc	gtt	gcc	cag	gac	ctg		692
Glu	Glu	Leu	Asn	Gly	Ala	Tyr	Lys	Ala	Ile	Pro	Val	Ala	Gln	Asp	Leu		
			185					190						195			
aac	gcg	cct	tct	gat	tgg	gac	agc	cgt	ggg	aag	gac	agt	tat	gaa	acg		740
Asn	Ala	Pro	Ser	Asp	Trp	Asp	Ser	Arg	Gly	Lys	Asp	Ser	Tyr	Glu	Thr		
		200					205						210				
agt	cag	ctg	gat	gac	cag	agt	gct	gaa	acc	cac	agc	cac	aag	cag	tcc		788
Ser	Gln	Leu	Asp	Asp	Gln	Ser	Ala	Glu	Thr	His	Ser	His	Lys	Gln	Ser		
	215					220					225						
aga	tta	tat	aag	cgg	aaa	gcc	aat	gat	gag	agc	aat	gag	cat	tcc	gat		836
Arg	Leu	Tyr	Lys	Arg	Lys	Ala	Asn	Asp	Glu	Ser	Asn	Glu	His	Ser	Asp		
230					235					240					245		
gtg	att	gat	agt	cag	gaa	ctt	tcc	aaa	gtc	agc	cgt	gaa	ttc	cac	agc		884
Val	Ile	Asp	Ser	Gln	Glu	Leu	Ser	Lys	Val	Ser	Arg	Glu	Phe	His	Ser		
				250					255					260			
cat	gaa	ttt	cac	agc	cat	gaa	gat	atg	ctg	gtt	gta	gac	ccc	aaa	agt		932
His	Glu	Phe	His	Ser	His	Glu	Asp	Met	Leu	Val	Val	Asp	Pro	Lys	Ser		
			265					270					275				
aag	gaa	gaa	gat	aaa	cac	ctg	aaa	ttt	cgt	att	tct	cat	gaa	tta	gat		980
Lys	Glu	Glu	Asp	Lys	His	Leu	Lys	Phe	Arg	Ile	Ser	His	Glu	Leu	Asp		
			280					285					290				
agt	gca	tct	tct	gag	gtc	aat	taaaaggaga	aaaaatacaa	tttctcactt								1031
Ser	Ala	Ser	Ser	Glu	Val	Asn											
	295					300											
tgcat	tttagt	caaaagaaaa	aatgctttat	agcaaaatga	aagagaacat	gaaatgcttc											1091
tttctcagtt	tattgggttg	atgtgtatct	atttgagtct	ggaaataact	aatgtgtttg												1151
ataattagtt	tagtttgttg	cttcatggaa	actccctgta	aactaaaagc	ttcagggtta												1211
tgtctatggt	cattctatag	aagaaatgca	aactatcact	gtattttta	atttgttatt												1271
ctctcatgaa	tagaaattta	tgtagaagca	aacaaaatac	ttttaccac	ttaaaaagag												1331
aatataacat	tttatgtcac	tataatcttt	tgttttttta	gttagtgtat	attttgttgt												1391
gattatcttt	ttgtgggtgtg	aataaatctt	ttatcttgaa	tgtaataaga	aaaaaaaaaa												1451
aaaaacaaaa	aaaaaaaaa																1469

<210> 2  
 <211> 300  
 <212> PRT  
 <213> Homo Sapien

&lt;400&gt; 2

Met Arg Ile Ala Val Ile Cys Phe Cys Leu Leu Gly Ile Thr Cys Ala  
 1 5 10 15

Ile Pro Val Lys Gln Ala Asp Ser Gly Ser Ser Glu Glu Lys Gln Leu  
 20 25 30

Tyr Asn Lys Tyr Pro Asp Ala Val Ala Thr Trp Leu Asn Pro Asp Pro  
 35 40 45

Ser Gln Lys Gln Asn Leu Leu Ala Pro Gln Thr Leu Pro Ser Lys Ser  
 50 55 60

Asn Glu Ser His Asp His Met Asp Asp Met Asp Asp Glu Asp Asp Asp  
 65 70 75 80

Asp His Val Asp Ser Gln Asp Ser Ile Asp Ser Asn Asp Ser Asp Asp  
 85 90 95

Val Asp Asp Thr Asp Asp Ser His Gln Ser Asp Glu Ser His His Ser  
 100 105 110

Asp Glu Ser Asp Glu Leu Val Thr Asp Phe Pro Thr Asp Leu Pro Ala  
 115 120 125

Thr Glu Val Phe Thr Pro Val Val Pro Thr Val Asp Thr Tyr Asp Gly  
 130 135 140

Arg Gly Asp Ser Val Val Tyr Gly Leu Arg Ser Lys Ser Lys Lys Phe  
 145 150 155 160

Arg Arg Pro Asp Ile Gln Tyr Pro Asp Ala Thr Asp Glu Asp Ile Thr  
 165 170 175

Ser His Met Glu Ser Glu Glu Leu Asn Gly Ala Tyr Lys Ala Ile Pro  
 180 185 190

Val Ala Gln Asp Leu Asn Ala Pro Ser Asp Trp Asp Ser Arg Gly Lys  
 195 200 205

Asp Ser Tyr Glu Thr Ser Gln Leu Asp Asp Gln Ser Ala Glu Thr His  
 210 215 220

Ser His Lys Gln Ser Arg Leu Tyr Lys Arg Lys Ala Asn Asp Glu Ser  
 225 230 235 240

Asn Glu His Ser Asp Val Ile Asp Ser Gln Glu Leu Ser Lys Val Ser

<400>	3																
acggcatcca	gtacagaggg	gctggacttg	gacccctgca	gcagccctgc	acaggagaag												60
cggcatataa	agccgcgctg	cccgggagcc	gctcggccac	gtccaccgga	gcatcctgca												120
ctgcagggcc	ggtctctcgc	tccagcagag	cctgcgcctt	tctgactcgg	tccggaacac												180
tgaaacaggt	catcactgca	tcttttttggc	aaaccaggag	ctcagctgca	ggaggcagg												239
atg	gtc	tgg	agg	ctg	gtc	ctg	ctg	gct	ctg	tgg	gtg	tgg	ccc	agc	acg		287
Met	Val	Trp	Arg	Leu	Val	Leu	Leu	Ala	Leu	Trp	Val	Trp	Pro	Ser	Thr		
1				5					10					15			
caa	gct	ggt	cac	cag	gac	aaa	gac	acg	acc	ttc	gac	ctt	ttc	agt	atc		335
Gln	Ala	Gly	His	Gln	Asp	Lys	Asp	Thr	Thr	Phe	Asp	Leu	Phe	Ser	Ile		
			20					25					30				
agc	aac	atc	aac	cgc	aag	acc	att	ggc	gcc	aag	cag	ttc	cgc	ggg	ccc		383
Ser	Asn	Ile	Asn	Arg	Lys	Thr	Ile	Gly	Ala	Lys	Gln	Phe	Arg	Gly	Pro		
		35					40					45					
gac	ccc	ggc	gtg	ccg	gct	tac	cgc	ttc	gtg	cgc	ttt	gac	tac	atc	cca		431
Asp	Pro	Gly	Val	Pro	Ala	Tyr	Arg	Phe	Val	Arg	Phe	Asp	Tyr	Ile	Pro		
	50					55					60						
ccg	gtg	aac	gca	gat	gac	ctc	agc	aag	atc	acc	aag	atc	atg	cgg	cag		479
Pro	Val	Asn	Ala	Asp	Asp	Leu	Ser	Lys	Ile	Thr	Lys	Ile	Met	Arg	Gln		
65					70					75					80		
aag	gag	ggc	ttc	ttc	ctc	acg	gcc	cag	ctc	aag	cag	gac	ggc	aag	tcc		527
Lys	Glu	Gly	Phe	Phe	Leu	Thr	Ala	Gln	Leu	Lys	Gln	Asp	Gly	Lys	Ser		
				85					90					95			
agg	ggc	acg	ctg	ttg	gct	ctg	gag	ggc	ccc	ggt	ctc	tcc	cag	agg	cag		575
Arg	Gly	Thr	Leu	Leu	Ala	Leu	Glu	Gly	Pro	Gly	Leu	Ser	Gln	Arg	Gln		
			100					105					110				
ttc	gag	atc	gtc	tcc	aac	ggc	ccc	gcg	gac	acg	ctg	gat	ctc	acc	tac		623
Phe	Glu	Ile	Val	Ser	Asn	Gly	Pro	Ala	Asp	Thr	Leu	Asp	Leu	Thr	Tyr		
		115					120					125					

tgg att gac ggc acc cgg cat gtg gtc tcc ctg gag gac gtc ggc ctg	671
Trp Ile Asp Gly Thr Arg His Val Val Ser Leu Glu Asp Val Gly Leu	
130 135 140	
gct gac tcg cag tgg aag aac gtc acc gtg cag gtg gct ggc gag acc	719
Ala Asp Ser Gln Trp Lys Asn Val Thr Val Gln Val Ala Gly Glu Thr	
145 150 155 160	
tac agc ttg cac gtg ggc tgc gac ctc ata gga cca gtt gct ctg gac	767
Tyr Ser Leu His Val Gly Cys Asp Leu Ile Gly Pro Val Ala Leu Asp	
165 170 175	
gag ccc ttc tac gag cac ctg cag gcg gaa aag agc cgg atg tac gtg	815
Glu Pro Phe Tyr Glu His Leu Gln Ala Glu Lys Ser Arg Met Tyr Val	
180 185 190	
gcc aaa ggc tct gcc aga gag agt cac ttc agg ggt ttg ctt cag aac	863
Ala Lys Gly Ser Ala Arg Glu Ser His Phe Arg Gly Leu Leu Gln Asn	
195 200 205	
gtc cac cta gtg ttt gaa aac tct gtg gaa gat att cta agc aag aag	911
Val His Leu Val Phe Glu Asn Ser Val Glu Asp Ile Leu Ser Lys Lys	
210 215 220	
ggt tgc cag caa ggc cag gga gct gag atc aac gcc atc agt gag aac	959
Gly Cys Gln Gln Gly Gln Gly Ala Glu Ile Asn Ala Ile Ser Glu Asn	
225 230 235 240	
aca gag acg ctg cgc ctg ggt ccg cat gtc acc acc gag tac gtg ggc	1007
Thr Glu Thr Leu Arg Leu Gly Pro His Val Thr Thr Glu Tyr Val Gly	
245 250 255	
ccc agc tcg gag agg agg ccc gag gtg tgc gaa cgc tcg tgc gag gag	1055
Pro Ser Ser Glu Arg Arg Pro Glu Val Cys Glu Arg Ser Cys Glu Glu	
260 265 270	
ctg gga aac atg gtc cag gag ctc tcg ggg ctc cac gtc ctc gtg aac	1103
Leu Gly Asn Met Val Gln Glu Leu Ser Gly Leu His Val Leu Val Asn	
275 280 285	
cag ctc agc gag aac ctc aag aga gtg tcg aat gat aac cag ttt ctc	1151
Gln Leu Ser Glu Asn Leu Lys Arg Val Ser Asn Asp Asn Gln Phe Leu	
290 295 300	
tgg gag ctc att ggt ggc cct cct aag aca agg aac atg tca gct tgc	1199
Trp Glu Leu Ile Gly Gly Pro Pro Lys Thr Arg Asn Met Ser Ala Cys	
305 310 315 320	
tgg cag gat ggc cgg ttc ttt gcg gaa aat gaa acg tgg gtg gtg gac	1247
Trp Gln Asp Gly Arg Phe Phe Ala Glu Asn Glu Thr Trp Val Val Asp	
325 330 335	
agc tgc acc acg tgt acc tgc aag aaa ttt aaa acc att tgc cac caa	1295
Ser Cys Thr Thr Cys Thr Cys Lys Lys Phe Lys Thr Ile Cys His Gln	
340 345 350	
atc acc tgc ccg cct gca acc tgc gcc agt cca tcc ttt gtg gaa ggc	1343
Ile Thr Cys Pro Pro Ala Thr Cys Ala Ser Pro Ser Phe Val Glu Gly	
355 360 365	
gaa tgc tgc cct tcc tgc ctc cac tcg gtg gac ggt gag gag ggc tgg	1391
Glu Cys Cys Pro Ser Cys Leu His Ser Val Asp Gly Glu Glu Gly Trp	
370 375 380	

tct	ccg	tgg	gca	gag	tgg	acc	cag	tgc	tcc	gtg	acg	tgt	ggc	tct	ggg	1439
Ser	Pro	Trp	Ala	Glu	Trp	Thr	Gln	Cys	Ser	Val	Thr	Cys	Gly	Ser	Gly	
385					390					395					400	
acc	cag	cag	aga	ggc	cgg	tcc	tgt	gac	gtc	acc	agc	aac	acc	tgc	ttg	1487
Thr	Gln	Gln	Arg	Gly	Arg	Ser	Cys	Asp	Val	Thr	Ser	Asn	Thr	Cys	Leu	
				405					410					415		
ggg	ccc	tcg	atc	cag	aca	cgg	gct	tgc	agt	ctg	agc	aag	tgt	gac	acc	1535
Gly	Pro	Ser	Ile	Gln	Thr	Arg	Ala	Cys	Ser	Leu	Ser	Lys	Cys	Asp	Thr	
			420					425					430			
cgc	atc	cgg	cag	gac	ggc	ggc	tgg	agc	cac	tgg	tca	cct	tgg	tct	tca	1583
Arg	Ile	Arg	Gln	Asp	Gly	Gly	Trp	Ser	His	Trp	Ser	Pro	Trp	Ser	Ser	
		435					440					445				
tgc	tct	gtg	acc	tgt	gga	gtt	ggc	aat	atc	aca	cgc	atc	cgt	ctc	tgc	1631
Cys	Ser	Val	Thr	Cys	Gly	Val	Gly	Asn	Ile	Thr	Arg	Ile	Arg	Leu	Cys	
	450					455					460					
aac	tcc	cca	gtg	ccc	cag	atg	ggg	ggc	aag	aat	tgc	aaa	ggg	agt	ggc	1679
Asn	Ser	Pro	Val	Pro	Gln	Met	Gly	Gly	Lys	Asn	Cys	Lys	Gly	Ser	Gly	
465					470					475					480	
cgg	gag	acc	aaa	gcc	tgc	cag	ggc	gcc	cca	tgc	cca	atc	gat	ggc	cgc	1727
Arg	Glu	Thr	Lys	Ala	Cys	Gln	Gly	Ala	Pro	Cys	Pro	Ile	Asp	Gly	Arg	
				485					490					495		
tgg	agc	ccc	tgg	tcc	ccg	tgg	tcg	gcc	tgc	act	gtc	acc	tgt	gcc	ggt	1775
Trp	Ser	Pro	Trp	Ser	Pro	Trp	Ser	Ala	Cys	Thr	Val	Thr	Cys	Ala	Gly	
			500					505					510			
ggg	atc	cgg	gag	cgc	acc	cgg	gtc	tgc	aac	agc	cct	gag	cct	cag	tac	1823
Gly	Ile	Arg	Glu	Arg	Thr	Arg	Val	Cys	Asn	Ser	Pro	Glu	Pro	Gln	Tyr	
		515					520					525				
gga	ggg	aag	gcc	tgc	gtg	ggg	gat	gtg	cag	gag	cgt	cag	atg	tgc	aac	1871
Gly	Gly	Lys	Ala	Cys	Val	Gly	Asp	Val	Gln	Glu	Arg	Gln	Met	Cys	Asn	
	530					535					540					
aag	agg	agc	tgc	ccc	gtg	gat	ggc	tgt	tta	tcc	aac	ccc	tgc	ttc	ccg	1919
Lys	Arg	Ser	Cys	Pro	Val	Asp	Gly	Cys	Leu	Ser	Asn	Pro	Cys	Phe	Pro	
545					550					555					560	
gga	gcc	cag	tgc	agc	agc	ttc	ccc	gat	ggg	tcc	tgg	tca	tgc	ggc	ttc	1967
Gly	Ala	Gln	Cys	Ser	Ser	Phe	Pro	Asp	Gly	Ser	Trp	Ser	Cys	Gly	Phe	
				565				570						575		
tgc	cct	gtg	ggc	ttc	ttg	ggc	aat	ggc	acc	cac	tgt	gag	gac	ctg	gac	2015
Cys	Pro	Val	Gly	Phe	Leu	Gly	Asn	Gly	Thr	His	Cys	Glu	Asp	Leu	Asp	
			580					585					590			
gag	tgt	gcc	ctg	gtc	ccc	gac	atc	tgc	ttc	tcc	acc	agc	aag	gtg	cct	2063
Glu	Cys	Ala	Leu	Val	Pro	Asp	Ile	Cys	Phe	Ser	Thr	Ser	Lys	Val	Pro	
		595					600					605				
cgc	tgt	gtc	aac	act	cag	cct	ggc	ttc	cac	tgc	ctg	ccc	tgc	ccg	ccc	2111
Arg	Cys	Val	Asn	Thr	Gln	Pro	Gly	Phe	His	Cys	Leu	Pro	Cys	Pro	Pro	
	610					615					620					
cga	tac	aga	ggg	aac	cag	ccc	gtc	ggg	gtc	ggc	ctg	gaa	gca	gcc	aag	2159
Arg	Tyr	Arg	Gly	Asn	Gln	Pro	Val	Gly	Val	Gly	Leu	Glu	Ala	Ala	Lys	
625					630					635					640	

acg gaa aag caa gtg tgt gag ccc gaa aac cca tgc aag gac aag aca	2207
Thr Glu Lys Gln Val Cys Glu Pro Glu Asn Pro Cys Lys Asp Lys Thr	
645 650 655	
cac aac tgc cac aag cac gcg gag tgc atc tac ctg ggt cac ttc agc	2255
His Asn Cys His Lys His Ala Glu Cys Ile Tyr Leu Gly His Phe Ser	
660 665 670	
gac ccc atg tac aag tgc gag tgc cag aca ggc tac gcg ggc gac ggg	2303
Asp Pro Met Tyr Lys Cys Glu Cys Gln Thr Gly Tyr Ala Gly Asp Gly	
675 680 685	
ctc atc tgc ggg gag gac tcg gac ctg gac ggc tgg ccc aac ctc aat	2351
Leu Ile Cys Gly Glu Asp Ser Asp Leu Asp Gly Trp Pro Asn Leu Asn	
690 695 700	
ctg gtc tgc gcc acc aac gcc acc tac cac tgc atc aag gat aac tgc	2399
Leu Val Cys Ala Thr Asn Ala Thr Tyr His Cys Ile Lys Asp Asn Cys	
705 710 715 720	
ccc cat ctg cca aat tct ggg cag gaa gac ttt gac aag gac ggg att	2447
Pro His Leu Pro Asn Ser Gly Gln Glu Asp Phe Asp Lys Asp Gly Ile	
725 730 735	
ggc gat gcc tgt gat gat gac gat gac aat gac ggt gtg acc gat gag	2495
Gly Asp Ala Cys Asp Asp Asp Asp Asp Asn Asp Gly Val Thr Asp Glu	
740 745 750	
aag gac aac tgc cag ctc ctc ttc aat ccc cgc cag gct gac tat gac	2543
Lys Asp Asn Cys Gln Leu Leu Phe Asn Pro Arg Gln Ala Asp Tyr Asp	
755 760 765	
aag gat gag gtt ggg gac cgc tgt gac aac tgc cct tac gtg cac aac	2591
Lys Asp Glu Val Gly Asp Arg Cys Asp Asn Cys Pro Tyr Val His Asn	
770 775 780	
cct gcc cag atc gac aca gac aac aat gga gag ggt gac gcc tgc tcc	2639
Pro Ala Gln Ile Asp Thr Asp Asn Asn Gly Glu Gly Asp Ala Cys Ser	
785 790 795 800	
gtg gac att gat ggg gac gat gtc ttc aat gaa cga gac aat tgt ccc	2687
Val Asp Ile Asp Gly Asp Asp Val Phe Asn Glu Arg Asp Asn Cys Pro	
805 810 815	
tac gtc tac aac act gac cag agg gac acg gat ggt gac ggt gtg ggg	2735
Tyr Val Tyr Asn Thr Asp Gln Arg Asp Thr Asp Gly Asp Gly Val Gly	
820 825 830	
gat cac tgt gac aac tgc ccc ctg gtg cac aac cct gac cag acc gac	2783
Asp His Cys Asp Asn Cys Pro Leu Val His Asn Pro Asp Gln Thr Asp	
835 840 845	
gtg gac aat gac ctt gtt ggg gac cag tgt gac aac aac gag gac ata	2831
Val Asp Asn Asp Leu Val Gly Asp Gln Cys Asp Asn Asn Glu Asp Ile	
850 855 860	
gat gac gac ggc cac cag aac aac cag gac aac tgc ccc tac atc tcc	2879
Asp Asp Asp Gly His Gln Asn Asn Gln Asp Asn Cys Pro Tyr Ile Ser	
865 870 875 880	
aac gcc aac cag gct gac cat gac aga gac ggc cag ggc gac gcc tgt	2927
Asn Ala Asn Gln Ala Asp His Asp Arg Asp Gly Gln Gly Asp Ala Cys	
885 890 895	

gac cct gat gat gac aac gat ggc gtc ccc gat gac agg gac aac tgc	2975
Asp Pro Asp Asp Asp Asn Asp Gly Val Pro Asp Asp Arg Asp Asn Cys	
900 905 910	
cgg ctt gtg ttc aac cca gac cag gag gac ttg gac ggt gat gga cgg	3023
Arg Leu Val Phe Asn Pro Asp Gln Glu Asp Leu Asp Gly Asp Gly Arg	
915 920 925	
ggt gat att tgt aaa gat gat ttt gac aat gac aac atc cca gat att	3071
Gly Asp Ile Cys Lys Asp Asp Phe Asp Asn Asp Asn Ile Pro Asp Ile	
930 935 940	
gat gat gtg tgt cct gaa aac aat gcc atc agt gag aca gac ttc agg	3119
Asp Asp Val Cys Pro Glu Asn Asn Ala Ile Ser Glu Thr Asp Phe Arg	
945 950 955 960	
aac ttc cag atg gtc ccc ttg gat ccc aaa ggg acc acc caa att gat	3167
Asn Phe Gln Met Val Pro Leu Asp Pro Lys Gly Thr Thr Gln Ile Asp	
965 970 975	
ccc aac tgg gtc att cgc cat caa ggc aag gag ctg gtt cag aca gcc	3215
Pro Asn Trp Val Ile Arg His Gln Gly Lys Glu Leu Val Gln Thr Ala	
980 985 990	
aac tcg gac ccc ggc atc gct gta ggt ttt gac gag ttt ggg tct gtg	3263
Asn Ser Asp Pro Gly Ile Ala Val Gly Phe Asp Glu Phe Gly Ser Val	
995 1000 1005	
gac ttc agt ggc aca ttc tac gta aac act gac cgg gac gac gac	3308
Asp Phe Ser Gly Thr Phe Tyr Val Asn Thr Asp Arg Asp Asp Asp	
1010 1015 1020	
tat gct ggc ttc gtc ttt ggt tac cag tca agc agc cgc ttc tat	3353
Tyr Ala Gly Phe Val Phe Gly Tyr Gln Ser Ser Ser Arg Phe Tyr	
1025 1030 1035	
gtg gtg atg tgg aag cag gtg acg cag acc tac tgg gag gac cag	3398
Val Val Met Trp Lys Gln Val Thr Gln Thr Tyr Trp Glu Asp Gln	
1040 1045 1050	
ccc acg cgg gcc tat ggc tac tcc ggc gtg tcc ctc aag gtg gtg	3443
Pro Thr Arg Ala Tyr Gly Tyr Ser Gly Val Ser Leu Lys Val Val	
1055 1060 1065	
aac tcc acc acg ggg acg ggc gag cac ctg agg aac gcg ctg tgg	3488
Asn Ser Thr Thr Gly Thr Gly Glu His Leu Arg Asn Ala Leu Trp	
1070 1075 1080	
cac acg ggg aac acg ccg ggg cag gtg cga acc tta tgg cac gac	3533
His Thr Gly Asn Thr Pro Gly Gln Val Arg Thr Leu Trp His Asp	
1085 1090 1095	
ccc agg aac att ggc tgg aag gac tac acg gcc tat agg tgg cac	3578
Pro Arg Asn Ile Gly Trp Lys Asp Tyr Thr Ala Tyr Arg Trp His	
1100 1105 1110	
ctg act cac agg ccc aag acc ggc tac atc aga gtc tta gtg cat	3623
Leu Thr His Arg Pro Lys Thr Gly Tyr Ile Arg Val Leu Val His	
1115 1120 1125	
gaa gga aaa cag gtc atg gca gac tca gga cct atc tat gac caa	3668
Glu Gly Lys Gln Val Met Ala Asp Ser Gly Pro Ile Tyr Asp Gln	
1130 1135 1140	



acc tac gct ggc ggg cgg ctg ggt cta ttt gtc ttc tct caa gaa	3713
Thr Tyr Ala Gly Gly Arg Leu Gly Leu Phe Val Phe Ser Gln Glu	
1145 1150 1155	
atg gtc tat ttc tca gac ctg aag tac gaa tgc aga gat att	3755
Met Val Tyr Phe Ser Asp Leu Lys Tyr Glu Cys Arg Asp Ile	
1160 1165 1170	
taaacaagat ttgctgcatt tccggcaatg cctgtgtcat gccatggtcc ctagacacct	3815
cagttcattg tggctccttg cggcttctctc tctagcagca cctcctgtcc cttgacctta	3875
actctgatgg ttcttcacct cctgccagca accccaaacc caagtgcctt cagaggataa	3935
atatcaatgg aactcagaga tgaacatcta acccactaga ggaaaccagt ttggtgatat	3995
atgagacttt atgtggagtg aaaattgggc atgocattac attgcttttt cttgtttgtt	4055
taaaaagaat gacgtttaca tataaaatgt aattacttat tgtatttatg tgtatatgga	4115
gttgaaggga atactgtgca taagccatta tgataaatta agcatgaaaa atattgctga	4175
actacttttg gtgcttaaag ttgtcactat tcttgaatta gagttgctct acaatgacac	4235
acaaatcccg ctaaataaat tataaacaag ggtcaattca aatttgaagt aatgttttag	4295
taaggagaga ttagaagaca acaggcatag caaatgacat aagctaccga ttaactaatc	4355
ggaacatgta aaacagttac aaaaataaac gaactctcct cttgtcctac aatgaaagcc	4415
ctcatgtgca gtagagatgc agtttcatca aagaacaaac atccttgcaa atgggtgtga	4475
cgcggttcca gatgtggatt tggcaaaacc tcatttaagt aaaagggttag cagagcaaag	4535
tgcggtgctt tagctgctgc ttgtgccgtt gtggcgctcg ggaggctcct gcctgagctt	4595
ccttccccag ctttgctgcc tgagaggaac cagagcagac gcacaggccg gaaaaggcgc	4655
atctaacgcg tatctaggct ttggtaactg cggacaagtt gcttttacct gatttgatga	4715
tacatttcat taaggttcca gttataaata ttttgttaat atttattaag tgactataga	4775
atgcaactcc atttaccagt aacttatttt aaatatgcct agtaacacat atgtagtata	4835
atttctagaa acaaacatct aataagtata taatcctgtg aaaatatgag gcttgataat	4895
attagggtgt cacgatgaag catgctagaa gctgtaacag aatacataga gaataatgag	4955
gagtttatga tggaacctta atatataatg ttgccagcga ttttagttca atatttgta	5015
ctgttatcta tctgctgtat atggaattct ttttaattcaa acgctgaaaa cgaatcagca	5075
tttagtcctg ccaggcacac ccaataatca gtcattgtga atatgcacaa gtttggtttt	5135
gtttttgttt tttttgttgg ttggtttttt tgctttaagt tgcattgatc ttctgcagga	5195
aatagtcact catcccactc cacataaggg gtttagtaag agaagtctgt ctgtctgatg	5255
atggataggg ggcaaactct tttccccttt ctgttaatag tcatcacatt tctatgcaa	5315
acaggaacga tccataactt tagtcttaat gtacacattg ctttttgata aaattaattt	5375
tggtgtttcc tttgaggttg atcgttgtgt tgtttgtctg cactttttac ttttttgcgt	5435

```

gtgggagctgt attcccgaga caacgaagcg ttgggatact tcattaaatg tagcgactgt      5495
caacagcgtg caggttttct gtttctgtgt tgtgggggtca accgtacaat ggtgtgggaa      5555
tgacgatgat gtgaatattt agaatgtacc atattttttg taaattattt atgttttttct      5615
aaacaaattt atcgtatagg ttgatgaaac gtcatgtgtt ttgccaaaga ctgtaaatat      5675
ttattttatgt gttcacatgg tcaaaatttc accactgaaa ccctgcactt agctagaacc      5735
tcatttttaa agattaacaa caggaaataa attgtaaaaa aggttttct      5784

```

```

<210> 4
<211> 1172
<212> PRT
<213> Homo Sapien

```

```

<400> 4

```

```

Met Val Trp Arg Leu Val Leu Leu Ala Leu Trp Val Trp Pro Ser Thr
1              5              10              15

```

```

Gln Ala Gly His Gln Asp Lys Asp Thr Thr Phe Asp Leu Phe Ser Ile
              20              25              30

```

```

Ser Asn Ile Asn Arg Lys Thr Ile Gly Ala Lys Gln Phe Arg Gly Pro
              35              40              45

```

```

Asp Pro Gly Val Pro Ala Tyr Arg Phe Val Arg Phe Asp Tyr Ile Pro
              50              55              60

```

```

Pro Val Asn Ala Asp Asp Leu Ser Lys Ile Thr Lys Ile Met Arg Gln
65              70              75              80

```

```

Lys Glu Gly Phe Phe Leu Thr Ala Gln Leu Lys Gln Asp Gly Lys Ser
              85              90              95

```

```

Arg Gly Thr Leu Leu Ala Leu Glu Gly Pro Gly Leu Ser Gln Arg Gln
              100              105              110

```

```

Phe Glu Ile Val Ser Asn Gly Pro Ala Asp Thr Leu Asp Leu Thr Tyr
              115              120              125

```

```

Trp Ile Asp Gly Thr Arg His Val Val Ser Leu Glu Asp Val Gly Leu
              130              135              140

```

```

Ala Asp Ser Gln Trp Lys Asn Val Thr Val Gln Val Ala Gly Glu Thr
145              150              155              160

```

```

Tyr Ser Leu His Val Gly Cys Asp Leu Ile Gly Pro Val Ala Leu Asp
              165              170              175

```

Glu Pro Phe Tyr Glu His Leu Gln Ala Glu Lys Ser Arg Met Tyr Val  
 180 185 190

Ala Lys Gly Ser Ala Arg Glu Ser His Phe Arg Gly Leu Leu Gln Asn  
 195 200 205

Val His Leu Val Phe Glu Asn Ser Val Glu Asp Ile Leu Ser Lys Lys  
 210 215 220

Gly Cys Gln Gln Gly Gln Gly Ala Glu Ile Asn Ala Ile Ser Glu Asn  
 225 230 235 240

Thr Glu Thr Leu Arg Leu Gly Pro His Val Thr Thr Glu Tyr Val Gly  
 245 250 255

Pro Ser Ser Glu Arg Arg Pro Glu Val Cys Glu Arg Ser Cys Glu Glu  
 260 265 270

Leu Gly Asn Met Val Gln Glu Leu Ser Gly Leu His Val Leu Val Asn  
 275 280 285

Gln Leu Ser Glu Asn Leu Lys Arg Val Ser Asn Asp Asn Gln Phe Leu  
 290 295 300

Trp Glu Leu Ile Gly Gly Pro Pro Lys Thr Arg Asn Met Ser Ala Cys  
 305 310 315 320

Trp Gln Asp Gly Arg Phe Phe Ala Glu Asn Glu Thr Trp Val Val Asp  
 325 330 335

Ser Cys Thr Thr Cys Thr Cys Lys Lys Phe Lys Thr Ile Cys His Gln  
 340 345 350

Ile Thr Cys Pro Pro Ala Thr Cys Ala Ser Pro Ser Phe Val Glu Gly  
 355 360 365

Glu Cys Cys Pro Ser Cys Leu His Ser Val Asp Gly Glu Glu Gly Trp  
 370 375 380

Ser Pro Trp Ala Glu Trp Thr Gln Cys Ser Val Thr Cys Gly Ser Gly  
 385 390 395 400

Thr Gln Gln Arg Gly Arg Ser Cys Asp Val Thr Ser Asn Thr Cys Leu  
 405 410 415

Gly Pro Ser Ile Gln Thr Arg Ala Cys Ser Leu Ser Lys Cys Asp Thr  
 420 425 430

Arg Ile Arg Gln Asp Gly Gly Trp Ser His Trp Ser Pro Trp Ser Ser  
 435 440 445  
 Cys Ser Val Thr Cys Gly Val Gly Asn Ile Thr Arg Ile Arg Leu Cys  
 450 455 460  
 Asn Ser Pro Val Pro Gln Met Gly Gly Lys Asn Cys Lys Gly Ser Gly  
 465 470 475 480  
 Arg Glu Thr Lys Ala Cys Gln Gly Ala Pro Cys Pro Ile Asp Gly Arg  
 485 490 495  
 Trp Ser Pro Trp Ser Pro Trp Ser Ala Cys Thr Val Thr Cys Ala Gly  
 500 505 510  
 Gly Ile Arg Glu Arg Thr Arg Val Cys Asn Ser Pro Glu Pro Gln Tyr  
 515 520 525  
 Gly Gly Lys Ala Cys Val Gly Asp Val Gln Glu Arg Gln Met Cys Asn  
 530 535 540  
 Lys Arg Ser Cys Pro Val Asp Gly Cys Leu Ser Asn Pro Cys Phe Pro  
 545 550 555 560  
 Gly Ala Gln Cys Ser Ser Phe Pro Asp Gly Ser Trp Ser Cys Gly Phe  
 565 570 575  
 Cys Pro Val Gly Phe Leu Gly Asn Gly Thr His Cys Glu Asp Leu Asp  
 580 585 590  
 Glu Cys Ala Leu Val Pro Asp Ile Cys Phe Ser Thr Ser Lys Val Pro  
 595 600 605  
 Arg Cys Val Asn Thr Gln Pro Gly Phe His Cys Leu Pro Cys Pro Pro  
 610 615 620  
 Arg Tyr Arg Gly Asn Gln Pro Val Gly Val Gly Leu Glu Ala Ala Lys  
 625 630 635 640  
 Thr Glu Lys Gln Val Cys Glu Pro Glu Asn Pro Cys Lys Asp Lys Thr  
 645 650 655  
 His Asn Cys His Lys His Ala Glu Cys Ile Tyr Leu Gly His Phe Ser  
 660 665 670  
 Asp Pro Met Tyr Lys Cys Glu Cys Gln Thr Gly Tyr Ala Gly Asp Gly  
 675 680 685

Leu Ile Cys Gly Glu Asp Ser Asp Leu Asp Gly Trp Pro Asn Leu Asn  
 690 695 700

Leu Val Cys Ala Thr Asn Ala Thr Tyr His Cys Ile Lys Asp Asn Cys  
 705 710 715 720

Pro His Leu Pro Asn Ser Gly Gln Glu Asp Phe Asp Lys Asp Gly Ile  
 725 730 735

Gly Asp Ala Cys Asp Asp Asp Asp Asp Asn Asp Gly Val Thr Asp Glu  
 740 745 750

Lys Asp Asn Cys Gln Leu Leu Phe Asn Pro Arg Gln Ala Asp Tyr Asp  
 755 760 765

Lys Asp Glu Val Gly Asp Arg Cys Asp Asn Cys Pro Tyr Val His Asn  
 770 775 780

Pro Ala Gln Ile Asp Thr Asp Asn Asn Gly Glu Gly Asp Ala Cys Ser  
 785 790 795 800

Val Asp Ile Asp Gly Asp Asp Val Phe Asn Glu Arg Asp Asn Cys Pro  
 805 810 815

Tyr Val Tyr Asn Thr Asp Gln Arg Asp Thr Asp Gly Asp Gly Val Gly  
 820 825 830

Asp His Cys Asp Asn Cys Pro Leu Val His Asn Pro Asp Gln Thr Asp  
 835 840 845

Val Asp Asn Asp Leu Val Gly Asp Gln Cys Asp Asn Asn Glu Asp Ile  
 850 855 860

Asp Asp Asp Gly His Gln Asn Asn Gln Asp Asn Cys Pro Tyr Ile Ser  
 865 870 875 880

Asn Ala Asn Gln Ala Asp His Asp Arg Asp Gly Gln Gly Asp Ala Cys  
 885 890 895

Asp Pro Asp Asp Asp Asn Asp Gly Val Pro Asp Asp Arg Asp Asn Cys  
 900 905 910

Arg Leu Val Phe Asn Pro Asp Gln Glu Asp Leu Asp Gly Asp Gly Arg  
 915 920 925

Gly Asp Ile Cys Lys Asp Asp Phe Asp Asn Asp Asn Ile Pro Asp Ile  
 930 935 940

Asp Asp Val Cys Pro Glu Asn Asn Ala Ile Ser Glu Thr Asp Phe Arg  
 945 950 955 960

Asn Phe Gln Met Val Pro Leu Asp Pro Lys Gly Thr Thr Gln Ile Asp  
 965 970 975

Pro Asn Trp Val Ile Arg His Gln Gly Lys Glu Leu Val Gln Thr Ala  
 980 985 990

Asn Ser Asp Pro Gly Ile Ala Val Gly Phe Asp Glu Phe Gly Ser Val  
 995 1000 1005

Asp Phe Ser Gly Thr Phe Tyr Val Asn Thr Asp Arg Asp Asp Asp  
 1010 1015 1020

Tyr Ala Gly Phe Val Phe Gly Tyr Gln Ser Ser Ser Arg Phe Tyr  
 1025 1030 1035

Val Val Met Trp Lys Gln Val Thr Gln Thr Tyr Trp Glu Asp Gln  
 1040 1045 1050

Pro Thr Arg Ala Tyr Gly Tyr Ser Gly Val Ser Leu Lys Val Val  
 1055 1060 1065

Asn Ser Thr Thr Gly Thr Gly Glu His Leu Arg Asn Ala Leu Trp  
 1070 1075 1080

His Thr Gly Asn Thr Pro Gly Gln Val Arg Thr Leu Trp His Asp  
 1085 1090 1095

Pro Arg Asn Ile Gly Trp Lys Asp Tyr Thr Ala Tyr Arg Trp His  
 1100 1105 1110

Leu Thr His Arg Pro Lys Thr Gly Tyr Ile Arg Val Leu Val His  
 1115 1120 1125

Glu Gly Lys Gln Val Met Ala Asp Ser Gly Pro Ile Tyr Asp Gln  
 1130 1135 1140

Thr Tyr Ala Gly Gly Arg Leu Gly Leu Phe Val Phe Ser Gln Glu  
 1145 1150 1155

Met Val Tyr Phe Ser Asp Leu Lys Tyr Glu Cys Arg Asp Ile  
 1160 1165 1170

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
7 February 2002 (07.02.2002)

PCT

(10) International Publication Number  
**WO 02/009735 A3**

(51) International Patent Classification<sup>7</sup>: **A61K 38/00**

(21) International Application Number: PCT/US01/24147

(22) International Filing Date: 31 July 2001 (31.07.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/222,071 1 August 2000 (01.08.2000) US

(71) Applicant (for all designated States except US): **UNIVERSITY OF WASHINGTON** [US/US]; Office of Technology Transfer, Suite 200, 1107 NE 45th Street, Seattle, WA 98105-4631 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **BORNSTEIN,**

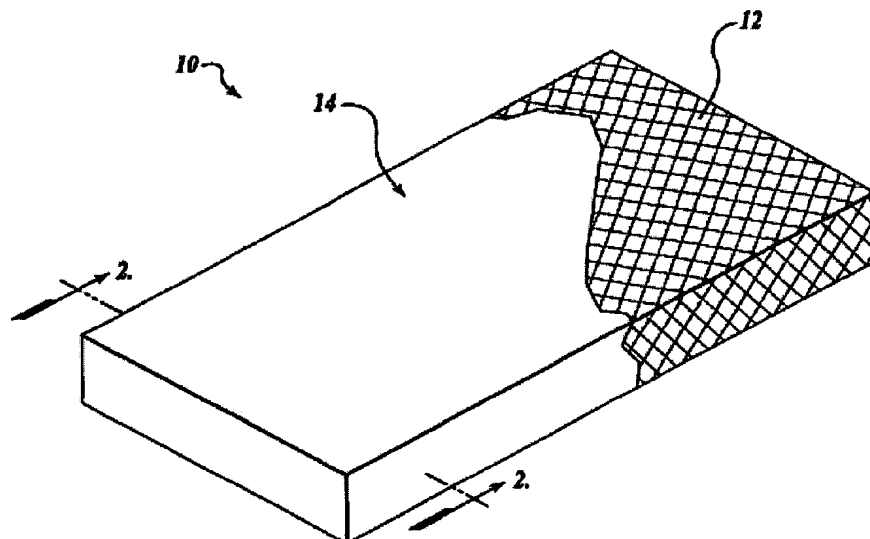
**Paul** [US/US]; 602 - 34th Avenue, Seattle, WA 98112 (US). **KYRIAKIDES, Themis** [CY/US]; 7729 - 45th Avenue S.W., Seattle, WA 98136 (US). **RATNER, Buddy** [US/US]; 1612 Bigelow Avenue N., Seattle, WA 98109 (US). **GIACHELLI, Cecilia** [US/US]; 3012 154th Street, S.E., Mill Creek, WA 98012 (US). **MARTINSON, Laura** [US/US]; Dexcon Inc., 6725 Meso Ridge Road, San Diego, CA 92121 (US). **SCATENA, Marta** [US/US]; 1825 N. 51st Street, Seattle, WA (US).

(74) Agent: **McGURL, Barry, F.**; Christensen O'Connor Johnson & Kindness PLLC Suite 2800, 1420 Fifth Avenue, Seattle, WA 98101 (US).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

[Continued on next page]

(54) Title: METHODS AND DEVICES TO MODULATE THE WOUND RESPONSE



(57) Abstract: In one aspect, the invention provides methods of modulating the amount and/or biological activity of thrombospondin (2) or osteopontin in an animal. The methods comprise the step of introducing into the animal an amount of osteopontin, and/or a thrombospondin (2) antagonist, effective to modulate the amount or biological activity of thrombospondin (2) or osteopontin in the animal. In another aspect, the invention provides medical devices comprising (a) a device body; and (b) a surface layer attached to the device body, the surface layer including an amount of an agents or antagonist of a matricellular protein sufficient to reduce the foreign body response against the medical device, wherein the medical device is adapted to be affixed to, or implanted within, the soft tissue of an animal.



**WO 02/009735 A3**



**(84) Designated States (regional):** ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**(88) Date of publication of the international search report:**  
3 October 2002

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

**Published:**

— with international search report



## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/24147

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K38/39 C07K14/78 A61K31/713 A61K39/395 A61L27/40  
 //A61P17/02

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, MEDLINE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 33415 A (GLANCY TODD P ;PETERSON DALE R (US); DEPUY ORTHOPAEDICS INC (US);) 8 July 1999 (1999-07-08)	1,11,12, 15, 19-22,27
Y	the whole document --- -/--	17,24



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

° Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*&\* document member of the same patent family

Date of the actual completion of the international search

4 July 2002

Date of mailing of the International search report

17/07/2002

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
 NL - 2280 HV Rijswijk  
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
 Fax: (+31-70) 340-3016

Authorized officer

Andres, S

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/24147

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>KYRIAKIDES THEMIS R ET AL: "Mice that lack the angiogenesis inhibitor, thrombospondin 2, mount an altered foreign body reaction characterized by increased vascularity"</p> <p>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 96, no. 8, 13 April 1999 (1999-04-13), pages 4449-4454, XP002184958 ISSN: 0027-8424 abstract page 4453, right-hand column, last paragraph -page 4454, last line</p> <p>---</p>	1-10, 12-27
Y	<p>KYRIAKIDES THEMIS R ET AL: "Accelerated wound healing in mice with a disruption of the thrombospondin 2 gene."</p> <p>JOURNAL OF INVESTIGATIVE DERMATOLOGY, vol. 113, no. 5, November 1999 (1999-11), pages 782-787, XP002204599 ISSN: 0022-202X the whole document</p> <p>---</p>	1-10, 12-16, 18-23, 25-27
A	<p>LIAW LUCY ET AL: "Altered wound healing in mice lacking a functional osteopontin gene (spp1)."</p> <p>JOURNAL OF CLINICAL INVESTIGATION, vol. 101, no. 7, 1 April 1998 (1998-04-01), pages 1468-1478, XP002204600 ISSN: 0021-9738 cited in the application</p> <p>---</p>	
A	<p>PULEO D A ET AL: "Understanding and controlling the bone-implant interface."</p> <p>BIOMATERIALS, vol. 20, no. 23-24, December 1999 (1999-12), pages 2311-2321, XP002204602 ISSN: 0142-9612</p> <p>---</p>	
T	<p>BORNSTEIN PAUL ET AL: "Thrombospondin 2, a matricellular protein with diverse functions."</p> <p>MATRIX BIOLOGY, vol. 19, no. 7, December 2000 (2000-12), pages 557-568, XP002204601 ISSN: 0945-053X cited in the application</p> <p>---</p>	

-/--

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/24147

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	KYRIAKIDES T R ET AL: "Regulation of angiogenesis and matrix remodeling by localized, matrix-mediated antisense gene delivery." MOLECULAR THERAPY: THE JOURNAL OF THE AMERICAN SOCIETY OF GENE THERAPY, vol. 3, no. 6, June 2001 (2001-06), pages 842-849, XP002204603 ISSN: 1525-0016 the whole document -----	1-7, 12-23,27
P,X	WO 00 57899 A (GEN HOSPITAL CORP ;DETMAR MICHAEL (US); STREIT MICHAEL (US)) 5 October 2000 (2000-10-05) page 9, line 14 - last line page 27, line 1 - line 19 page 42, line 16 -page 46 page 54, line 18 -page 55 claims 26,36-43 -----	1-10,12, 13,15,16

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 01/24147

### Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
  
Although claims 1-18 and 27 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

#### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/24147

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9933415	A	08-07-1999	AU 2021499 A	19-07-1999
			EP 1043963 A1	18-10-2000
			JP 2001527030 T	25-12-2001
			WO 9933415 A1	08-07-1999
<hr/>				
WO 0057899	A	05-10-2000	AU 3917200 A	16-10-2000
			EP 1171151 A1	16-01-2002
			WO 0057899 A1	05-10-2000
			US 2002022592 A1	21-02-2002